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STUDIES ON THE AQUEOUS HUMOUR

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The aqueous humour may be considered to be *a*, an ultra-filtrate of plasma in thermodynamic equilibrium with it; *b*, a secretion of the cells of the epithelial lining of the eye, or *c*, a fluid which owes its origin to an initial filtration process, but whose composition is modified by the secretion into, or the absorption from it of certain constituents. The experimental evidence in relation to these theories has been discussed at length by Davson and Quilliam (1940) and by Duke-Elder, Quilliam and Davson (1940) and it was suggested that the last named hypothesis was most consistent with the facts, especially in view of the divergence of the experimentally determined distribution of chloride between the aqueous humour and plasma (Hodgson, 1938) and of the osmotic pressures of the two fluids (Benham, Duke-Elder and Hodgson, 1938; Roepke and Hetherington, 1940) from those required on the basis of thermodynamic equilibrium. This equilibrium demands a distribution ratio, $R_{Cl} = [Cl]_{Serum}/[Cl]_{Aqueous}$ equal to 0.96 (Van Slyke, 1926) whereas Hodgson reported an average value for dogs of 0.92[1], and it might be thought that sodium chloride is being secreted into the aqueous humour so that the chloride concentration is greater in this fluid than the equilibrium demands. In this case the distribution of sodium should also be divergent from theory; however Davson (1939) has shown by methods accurate to within 1 in 500 that in the cat the mean distribution ratio, $R_{Na} = [Na]_{Serum}/[Na]_{Aqueous}$ is 1.03, i.e., close to the theoretical one of 1.04, and that the mean chloride distribution ratio was 0.945, and therefore not so divergent as in the dog.

In the present work the distribution of sodium has been examined in the dog (a more suitable experimental animal than the cat since it is possible to remove eye fluids with only local anesthesia); further, the distribu-

[1] Hodgson does not report the protein contents of his sera, so that we have calculated this ratio on the assumption of a mean value of 7 per cent.

tion of chloride has been investigated under several experimental conditions, and finally the ability, or otherwise, of the eye membranes to exclude inulin, injected into the blood, from penetrating the eye fluid has been tested. These last experiments were carried out to see whether it is likely that these membranes can have any effective secretory activity. Inulin has a molecular weight of 5000, and being a polysaccharide has a large number of water-soluble groups in it, and may consequently be expected to diffuse through a membrane, if at all, by way of the inter-cellular spaces. If these spaces are large enough to permit its passage through them, it is very unlikely that the cells constituting the epithelial lining of the eye would be able to secrete substances into the eye at rates great enough to maintain appreciable concentration gradients over a period of time, owing to the rapidity with which the secreted substances would escape back to the blood stream through these large inter-cellular spaces.[2]

EXPERIMENTAL. In the studies on sodium distribution the aqueous humour was drawn from the cocainised eye without other anesthesia; the blood was drawn by puncture of the femoral vein. During the studies on the chloride distribution and inulin penetration it was found that essentially similar results were obtained with and without general anesthesia, so generally the dog was given sodium amytal intravenously. In the studies on the penetration of inulin, the concentration of this substance in the blood was raised to about 100 mgm. per cent by a single venous injection of 10 per cent inulin in isotonic NaCl, and then maintained at about that level by continuous intravenous injection with the same solution. The chemical methods for the determination of sodium and chloride were those described earlier (Davson, 1939); inulin was determined by estimating the total reducing value by the Hagedorn-Jensen (1923) method before and after hydrolysis with 0.1 N H_2SO_4. Excellent reproducibility (within the titration error of 2 per cent) was obtained by this method on samples of 0.2 ml. of fluid.

RESULTS. *Distribution of sodium.* In table 1 the concentrations of sodium, expressed in millimoles per kilogram H_2O, in the serum and aqueous humour of ten separate dogs are shown, giving a mean value of R_{Na} of 1.04, in excellent agreement with the theoretical value deduced by Van Slyke (1926) on the basis of an average base-binding power of plasma proteins. We may conclude from these results that the distribution of chloride, described by Hodgson, is not due to the secretion of extra sodium chloride into a filtrate of blood plasma, since if this occurred the value of R_{Na} would be 1.00 or less.

Distribution of chloride. Preliminary experiments confirmed Hodgson's observation that the value of R_{Cl} was less than 0.96 (the mean of 21 of

[2] We are indebted to Prof. F. R. Winton of University College, London, for suggesting this mode of approach.

the experiments carried out during this work gave a value of 0.93), and a variety of experiments have been performed to determine whether the result is an artifact of the experimental procedure; the detailed description of many of the results would be tedious, so some will be merely indicated.

a. The discrepant ratio is not due to the instillation of cocaine hydrochloride into the eye, since no difference in the concentrations of chloride in the aqueous humours of the two eyes of a dog was observed when only one eye was instilled with cocaine.

b. The discrepant ratio is not due to transient variations in the blood chloride, since experiments in which the blood chloride was followed for several hours before the removal of the aqueous humour showed only small

TABLE 1

Comparison of the concentrations of sodium in blood serum and aqueous humour of dogs

EXPERIMENT	SERUM	AQUEOUS HUMOUR	PER CENT SOLIDS IN SERUM	SERUM	AQUEOUS HUMOUR	R_{Na}
	millimols/kgm.	*millimols/kgm.*		*millimols/kgm. H_2O*	*millimols/kgm. H_2O*	
1	140.2	145.9	8.5	153.1	147.3	1.040
2	141.4	144.1	6.9	151.8	145.5	1.045
3	134.1	136.5	6.4	143.2	137.8	1.040
4	141.4	145.7	9.0	155.3	147.2	1.055
5	139.5	147.6	8.0	151.7	149.1	1.020
6	139.7	144.7	9.0	153.6	146.2	1.050
7	137.4	140.7	6.5	147.0	142.1	1.035
8	137.7	142.5	8.9	151.1	144.0	1.050
9	139.7	144.5	7.4	150.9	146.0	1.035
10	139.1	144.1	8.6	152.3	145.5	1.045
Mean						1.040
S.D.						0.01
S.E.						0.003

variations, and these showed no definite trend from one experiment to another.

c. The discrepant ratio is not due to the metabolic activity of the lens, since the removal of this body from the eye and subsequent determination of the chloride ratios in the two eyes separately gave, with some animals, identical chloride ratios in the two eyes The results of these experiments, in which the chloride ratios were followed as long as three months after the removal of the lens, are shown in table 2, and it may be seen that in experiment 1, after three months the concentration of chloride was the same in both eyes; similarly in experiment 2 after two months. In general the impression was gained that during the first month the concentration of chloride in the aphakic eye was less than in the normal (expts. 1, 2 and 3) but that the concentrations tended to become equal later.

The effect of paracentesis. After removal of the aqueous humour the eye refills rapidly, so that within 15 minutes sufficient of the new fluid can be drawn out and subjected to analysis. This fluid, unlike the normal aqueous humour, contains appreciable amounts of protein (a maximal concentration of 2 per cent was observed) which, however, soon disappears, so that within 8 hours the fluid is quite clear. If the aqueous humour is formed initially as a filtrate from plasma, and only subsequently subjected to secretory activity, the fluid reformed immediately after paracentesis may be expected to have a value of R_{Cl} characteristic of a filtrate of plasma, whilst the fluid withdrawn later, say within 12 hours, may be expected to have resumed its characteristic distribution ratio. In the following

TABLE 2

The effect of removal of the lens on the distribution of chloride between the blood and aqueous humour

EXPERIMENT NUMBER	FLUID	TIME AFTER REMOVAL OF LENS		
		1 month	2 months	3 months
		Concentration of chloride (mmols./kgm. H_2O)		
1	Normal aqueous	128.5	127.5	127.5
	Aphakic aqueous	127.0	126.0	127.5
	Serum	123.0	118.5	120.0
2	Normal aqueous	131.5	122.5	129.5
	Aphakic aqueous	127.0	125.5	131.0
	Serum	120.5	120.5	122.5
3	Normal aqueous	129.5	126.0	
	Aphakic aqueous	126.5	126.0	
	Serum	124.5	119.0	
4	Normal aqueous		127.5	
	Aphakic aqueous		129.0	
	Serum		119.0	

experiments aqueous humour was withdrawn from one eye, and at periods varying from 15 minutes to 24 hours the refilled aqueous humour and the normal humour from the other eye were withdrawn, together with a sample of the blood. The concentrations of chloride in the two fluids are shown in table 3, where the values of R_{Cl} for each eye are also shown. The experiments were all carried out on separate dogs. The table shows that within 15 minutes the value of R_{Cl} for the refilled eye is considerably greater than that for the normal eye (0.96 in comparison with 0.925); within eight hours the concentration of chloride in the two eyes is equal.[3]

[3] It may be argued that the presence of protein in the aqueous humour *per se* would increase the value of R_{Cl}; however it is unlikely that it would produce the large effects observed, and it is found that the effect on the sodium ratio is small.

Permeability to inulin. In these experiments the concentration of inulin in the blood was maintained at around 100 mgm. per cent for several hours and the aqueous humour was withdrawn from the two eyes at different intervals. In table 4 the results of a typical experiment are shown. (Normal plasma and aqueous humour invariably show an increase in the

TABLE 3

The effect of paracentesis on the distribution of chloride between the blood and aqueous humour of dogs

TIME AFTER PARACENTESIS	EYE	AQUEOUS HUMOUR CHLORIDE CONCN.	SERUM CHLORIDE CONCN.	R_{Cl}
		mmols./kgm. H_2O	*mmols./kgm. H_2O*	
15 minutes	Normal	126.0	116.5	0.925
	Refilled	121.5		0.960
2 hours	Normal	128.5	118.5	0.920
	Refilled	126.0		0.940
3 hours	Normal	126.0	118.5	0.940
	Refilled	124.0		0.955
5 hours	Normal	129.5	120.0	0.925
	Refilled	128.5		0.935
8 hours	Normal	127.0	115.4	0.910
	Refilled	127.0		0.910

TABLE 4

Changes in the reducing value, expressed as milligrams glucose per cent, of aqueous humour after injection of inulin into the dog

INTERVAL BETWEEN 1ST INJECTION OF INULIN AND WITHDRAWAL OF FLUID	FLUID	REDUCING VALUE BEFORE HYDROLYSIS	REDUCING VALUE AFTER HYDROLYSIS	REDUCING VALUE AFTER HYDROLYSIS CORRECTED	INULIN
3 hours	Left aqueous	95.5	105.5	98.5	3.0
6 hours	Right aqueous	91.5	102.0	95.0	3.5
30 minutes	Blood serum	117.5	260.0	253.0	135.5
3 hours	Blood serum	107.0	190.0	187.0	80.0
6 hours	Blood serum	107.0	228.0	221.0	114.0

reducing value after hydrolysis, generally of the order of 7 mgm. glucose per cent, and the values obtained have been corrected for this, as is shown in the table.) The table shows a small increase in the reducing value after hydrolysis in the aqueous humour, but since this is hardly greater in the six hour specimen than in the three hour one, it is unlikely that it can be ascribed to the penetration of inulin into the eye.

Discussion. The results on the distribution of chloride between the aqueous humour and serum suggest that the aqueous humour is not formed by passive filtration alone, because the ratio is too low, having a mean value in 21 experiments of 0.930 $\pm$ 0.006 (Standard Error). The rapid refilling of the eye after paracentesis, and the appearance of appreciable quantities of protein in the reformed fluid, suggest that in this case the reformed aqueous humour is simply a filtrate; such a view is supported by the observation that the chloride ratio in the fluid withdrawn 15 minutes after paracentesis is close to that required by the Donnan Equilibrium. The presence of protein in the reformed aqueous humour suggests that the rapid fall in intra-ocular pressure associated with paracentesis causes a stretching of the membranes separating the plasma from the eye fluids, increasing their inter-cellular spaces and thereby allowing some proteins to penetrate. (It may be mentioned here that inulin penetrates the eye rapidly after paracentesis.) In these circumstances it is to be expected that any secretory activity of the epithelium will be completely masked, owing to the large leaks in the inter-cellular spaces. With the repair of the leaks, which apparently occurs rapidly, the secretory activity may become effective and the chloride concentration attain its normal value in respect to that of plasma.

The fact that inulin does not penetrate the eye, although present in the plasma for as long as six hours, certainly suggests that one of the membranes, interposed between the capillary endothelium and the aqueous humour, is considerably more selective than the capillary endothelium itself, and this membrane is probably the epithelial lining of the vascularised structures of the eye, namely, the iris, ciliary body and choroid. The permeability of such complex membranes has been discussed by Chambers (1940) who has shown that their selectivity in regard to water soluble substances like sugars is largely determined by the packing of the individual cells and the nature of the inter-cellular cement. The impermeability of the eye membrane to inulin differentiates the latter from the capillary endothelium which is freely permeable to this substance. The further differentiation between the more closely packed intestinal epithelium type of membrane and the still more closely packed one characterised by the kidney tubular epithelium awaits further studies along the lines of the inulin experiments described here.

In conclusion we may state that although the bulk of the evidence regarding the nature of the aqueous humour suggests that it is formed simply as a passive filtrate from plasma, and consequently that the intra-ocular pressure will be determined largely by the simple physical factors of colloid osmotic pressure of the plasma and hydrostatic capillary pressure, other evidence suggests that super-imposed on this primary process of filtration there is a certain selective activity on the part of the cellular lining

of the eye. Whether or not this has any obvious functional significance in regard to the maintenance of the intra-ocular pressure cannot yet be decided; the fact that the extra chloride in the aqueous humour is not accompanied by extra sodium (potassium and calcium are also ruled out by the work of Davson, Duke-Elder and Benham, 1936, and of Stary and Winternitz, 1932) suggests that it is compensated by a deficiency of some other anion or alternatively that there is an excess of some organic cation in the aqueous humour.

SUMMARY

The distribution of sodium and chloride between the aqueous humour and blood plasma of dogs has been investigated under normal and experimental conditions and it has been concluded that although filtration represents the primary process in aqueous humour formation, super-imposed on this process there is evidence of some secretion. It has been shown that inulin cannot pass from the blood into the eye.

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THE EFFECT OF THE PITUITARY ADRENOCORTICOTROPIC HORMONE AND OF CORTICOSTERONE ACETATE ON INSULIN HYPOGLYCEMIA AND LIVER GLYCOGEN IN ADRENALECTOMIZED MICE

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It was first demonstrated by Houssay and Potick (1) and by Benedetto (2) that the administration of anterior pituitary extracts to normal or hypophysectomized animals inhibits the hypoglycemic action of subsequently injected insulin. These findings have since been substantiated by different investigators. Young (3, 4) has investigated the anti-insulin action of various anterior pituitary preparations and proposed the term "glycotropic" for this effect. He found that the pituitary substance which inhibits the hypoglycemic action of insulin was different from prolactin, the glycotropic factor, and the gonadotropic hormones. Jensen and Grattan (5) reported the results of studies in normal mice indicating that the anti-insulin[1] effect of the anterior pituitary may be attributed to the adrenocorticotropic principle of that gland and that the effect is mediated through the adrenal cortex. Grattan and Jensen (6) have demonstrated that apparently only those principles of the adrenal cortex substituted in ring 3 (keto- or hydroxy group) exert this effect. This finding is in agreement with the observations of various other investigators (7, 8, 9, 10), that apparently only those adrenal cortical principles, which contain either a keto- or hydroxy group at C_{11}, exert a significant influence on carbohydrate metabolism. Grattan and Jensen (6) suggested that the anti-insulin response produced by the adrenocorticotropic hormone and the corticosterone-like compounds is probably due to the ability of these substances to promote the formation of liver glycogen. Hartman and his associates (11) have likewise concluded that the increased resistance to insulin of normal fasted mice after cortin injections is apparently due to gluconeogenesis, since both the blood sugar and liver glycogen are elevated.

[1] The term "anti-insulin" as employed by us refers only to the ability of a substance to counteract the hypoglycemia subsequent upon the injection of insulin into an animal. One has to distinguish between specific and unspecific effects. The unspecific effects will be discussed in another paper.

The anti-insulin response elicited by certain adrenocortical principles in normal mice may be used as an index of the adequacy of their effect on carbohydrate metabolism.

The object of this study was to extend the observations on normal animals by determining the effect of the adrenocorticotropic pituitary factor and of corticosterone on insulin hypoglycemia and liver glycogen in adrenalectomized mice under similar experimental conditions as previously employed (5, 6).

TABLE 1

Anti-insulin tests in normal and adrenalectomized mice

The corticosterone acetate was administered in peanut oil, 0.2 cc. containing 0.5 mgm. of the steroid; the adrenocorticotropic preparation was injected in aqueous solution of pH = 7.5, 0.5 cc. containing 5.0 mgm. of the hormone, all injections subcutaneously.

PREPARATION INJECTED	CONDITION OF ANIMALS	INSULIN DOSE PER KILO	TOTAL NUMBER OF ANIMALS	NUMBER OF CONVULSIONS	PER CENT CONVULSIONS
		units			
Controls*	Intact	1.5 or 2.0	202	179	89
5.0 mgm. adrenocorticotropic*	Intact	1.5 or 2.0	128	21	16
0.5 mgm. corticosterone acetate*†	Intact	1.5	13	0	0
Controls	Adrenalectomized	0.4	33	30	90
5.0 mgm. adrenocorticotropic‡	Adrenalectomized	0.4	16	14	88
0.5 mgm. corticosterone acetate†	Adrenalectomized	0.4	16	2	13

* These values were taken from table 1 of a previous publication (6).

† Supplied by Dr. E. C. Kendall.

‡ Same preparation as used in previous experiments (5, 6).

Experimental. Male mice weighing from 20 to 25 grams were adrenalectomized approximately 6 days before the experiments. During the postoperative period, they were maintained on a daily subcutaneous injection of 0.1 mgm. desoxycorticosterone acetate in 0.1 cc. of peanut oil and received 0.9 per cent sodium chloride in their drinking water.

Anti-insulin test. The procedure followed in determining the anti-insulin effect of the two hormones was the same as that previously employed by Jensen and Grattan (5) except for the following changes: *1.* Due to the greater insulin-sensitivity of adrenalectomized mice, 0.4 unit of insulin per kilo was found to be sufficient to produce a high percentage of convulsions, this dose represents one-fifth of the insulin dose employed

in the studies in normal mice (2 units per kilo). *2.* Before the 6 hour test period, all animals received their daily maintenance dose of desoxycorticosterone acetate. *3.* Controls for the corticosterone tests received peanut oil. *4.* During the 6 hour fast, the animals had access to 0.9 per cent sodium chloride in their drinking water.

The anti-insulin response of the two substances in adrenalectomized mice is recorded in table 1.

TABLE 2

Effect of pituitary adrenocorticotropic hormone and of corticosterone acetate on liver glycogen of normal and adrenalectomized mice

The corticosterone acetate was administered in peanut oil, 0.2 cc. containing 0.5 mgm. of the steroid; the adrenocorticotropic preparation was injected in aqueous solution of pH = 7.5, 0.5 cc. containing 5.0 mgm. of the hormone, all injections subcutaneously.

PREPARATION INJECTED	NUMBER OF ANIMALS	CONDITION OF ANIMALS	AVERAGE WEIGHT OF ANIMAL AT ONSET OF FAST	AVERAGE WEIGHT LOSS PER ANIMAL DURING FAST	AVERAGE LIVER WEIGHT PER ANIMAL	LIVER GLYCOGEN
			grams	*grams*	*grams*	*mgm. per cent*
Controls*	72	Intact	19.9	0.90	1.18	616
5.0 mgm. adrenocorticotropic*	28	Intact	20.3	0.23	1.32	2,346
0.5 mgm. corticosterone acetate*†	28	Intact	19.7	1.07	1.19	2,127
Controls	21	Adrenalectomized	20.9	0.67	1.28	440
5.0 mgm. adrenocorticotropic‡	25	Adrenalectomized	21.5	0.21	1.34	305
0.5 mgm. corticosterone acetate†	12	Adrenalectomized	21.8	0.85	1.47	3,415

* These values were taken from table 2 of a previous publication (6).

† Supplied by Dr. E. C. Kendall.

‡ Same preparation as used in previous experiments (5, 6).

Effect on liver glycogen. It has been found by Grattan and Jensen (6) that the adrenocorticotropic hormone as well as corticosterone and chemically related compounds greatly increase the liver glycogen of normal mice during a six hour fast, while desoxycorticosterone failed to do so. Both adrenocorticotropic hormone and corticosterone acetate were tested for their effect on liver glycogen in adrenalectomized mice according to the procedure employed by Grattan and Jensen (6). The animals received the same preliminary treatment as in the anti-insulin tests. The effectiveness of the two preparations in promoting the deposition of liver glycogen in adrenalectomized mice is illustrated in table 2.

Discussion. From the data presented in tables 1 and 2 it is evident that the adrenocorticotropic factor of the anterior pituitary did not exert any anti-insulin effect and also failed to promote the formation of liver glycogen in adrenalectomized mice. In normal mice a positive response has been observed as previously reported (6). Corticosterone exerted a pronounced anti-insulin response and also markedly increased the deposition of liver glycogen under identical experimental conditions. These findings are in agreement with our assumption that the anti-insulin effect of the anterior pituitary is mediated through the adrenal cortex and inhibits insulin hypoglycemia by promoting the formation of liver glycogen. They do not exclude, however, the possibility that other pituitary principles may modify insulin resistance and other phases of carbohydrate metabolism.

SUMMARY

It has been found that the adrenocorticotropic factor of the anterior pituitary failed to produce an anti-insulin effect and to promote the deposition of liver glycogen in adrenalectomized mice. On the other hand, administration of corticosterone was found to protect adrenalectomized mice against insulin hypoglycemia and to increase the amount of liver glycogen under the same experimental conditions. These results confirm our assumption that the anti-insulin effect of the anterior pituitary is produced by the adrenocorticotropic factor and is mediated through the adrenal cortex.

We wish to express our appreciation to Dr. E. C. Kendall of the Mayo Clinic for kindly supplying us with crystalline corticosterone acetate. We are also indebted to Dr. E. Schwenk of the Schering Corporation for supplying us with synthetic desoxycorticosterone acetate.

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CORTILACTIN, THE LACTATION FACTOR OF THE ADRENAL[1]

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Adequate lactation in the adrenalectomized animal can be maintained if certain types of adrenal extract are used (1). In 1933 Brownell, Lockwood and Hartman (2) showed that such an extract could be divided into two fractions, one which maintained an adrenalectomized mother rat in good condition but would not support lactation, while the other supported lactation but had little influence on the maintenance of an adrenalectomized animal. The active factor in the latter fraction was named cortilactin.

The effectiveness of cortilactin does not appear to be limited to the rat. Thorn and Hartman (3) obtained positive effects with it in the human being.

We have been able to separate cortilactin from whole adrenal tissue by iso-electric precipitation. We have also shown that cortilactin bears no relation to gluconeogenesis.

METHODS. *Preparation of cortilactin.* We started either with the lipid precipitate obtained as a by-product in the making of ordinary adrenal extract (4) or with adrenal tissue. In the first instance the glands were extracted with 95 per cent ethyl alcohol. The alcoholic extract was concentrated to $\frac{1}{15}$ volume. The resulting concentrate was extracted with ethyl ether, the aqueous residue being discarded. The ether solution was concentrated to small volume and the residue taken up in 70 per cent alcohol. On chilling this solution to $-12°$C. a lipid precipitate was thrown down. This is the lipid precipitate mentioned above. It is dissolved at room temperature in alkaline (pH 10) 70 per cent alcohol.

When cortilactin was prepared directly from the glands, they were extracted first with alkaline (pH 10) 70 per cent ethyl alcohol. This method is preferable because separation of cortilactin from the lipid precipitate may be difficult on account of emulsification.

From either alkaline 70 per cent alcoholic solution, cortilactin was separated according to the method of Riddle, Bates and Dykshorn (5) by iso-electric precipitation in 86 per cent alcohol at a pH 5.8. The precipitate

[1] Aided by grants from The National Research Council Committee on Research in Endocrinology and the Comly Fund of The Ohio State University.

was dissolved in water or saline for administration. Potency was increased by reprecipitation.

Methods for the preparation of adrenal extract, cortin and sodium factor are described elsewhere (6).

The prolactin was dissolved in saline. The desoxycorticosterone was dissolved in 5 per cent propylene glycol saline solution.

Lactation assay. Pigeon. The pigeon crop gland response is most satisfactory for the assay of cortilactin because of the limited amount of material needed and the relatively short time required. Certain features of the assay methods of Riddle and Braucher (7) and of Lyons (8) have been combined for this test. The subjects were one month old squabs. The injections were made twice daily into the pectoral muscle for four days. On the fifth day the crop was removed, freed from adhering fat, washed and dried at 105°C. for seven hours. An increase in weight over normal control values was a measure of the effect.

Rat. Adrenal preparations proven potent by the pigeon assay were tested for their ability to support lactation in the adrenalectomized rat. These animals were maintained on the special diet which Daggs (9) used in his lactation studies. Young pregnant rats were adrenalectomized one to five days before term, after which they were maintained in good condition by adrenal extract. Only those animals which produced a normal litter were used. At birth the number of pups was reduced to six. The first seventeen days *post partum* give a direct measure of milk secretion (9), therefore the observations were not extended beyond this period.

Rat test for gluconeogenesis. The gluconeogenetic power of cortilactin was tested in the rat (10). Twelve young males of about 150 grams in weight were fasted for 24 hours. Every hour for the last seven hours of the fast four of these animals were injected subcutaneously with 1.3 mgm. of cortilactin (second iso-electric precipitate) in 1 cc. of physiological saline. At the eighth hour of the fast the extract was given intraperitoneally. A second four were injected with equal quantities of physiological saline. At each injection, the third four were given 0.06 mgm. of corticosterone in 1 cc. of 5 per cent propylene glycol in physiological saline. One hour after the last injection the animals were anesthetized with nembutal and the livers rapidly excised and dropped into 30 per cent potassium hydroxide. Liver glycogen was determined by the method of Good, Kramer and Somogyi (11).

RESULTS. *Pigeon crop response. Controls.* The birds varied from 250 to 470 grams in weight. The dry crop did not necessarily parallel the body weight. Groups of normal birds were run with each test.

The dry crop weights of 36 normal birds ranged from 0.39 to 0.87 gram with an average of 0.61 gram.

Prolactin. Purified prolactin in doses of $\frac{1}{2}$ to 1 mgm. (1 mgm. contained

6 Riddle units) daily gave definite enlargement of the crop. Comparison of the effects of various preparations is shown in figure 1.

Adrenal preparations. A few birds were tested with unfractionated or whole adrenal extract, extract containing cortin, extract containing sodium factor and desoxycorticosterone. All were negative. Figure 1 shows the doses employed.

The first precipitate prepared from the 70 per cent alcoholic direct extraction of the adrenal tissue (called "glands" in fig.) was somewhat better than the first precipitate (called "lipid" in fig.) prepared from the lipid by-product of ordinary extract preparation. The potency was doubled by iso-electric reprecipitation (called "second" in fig.).

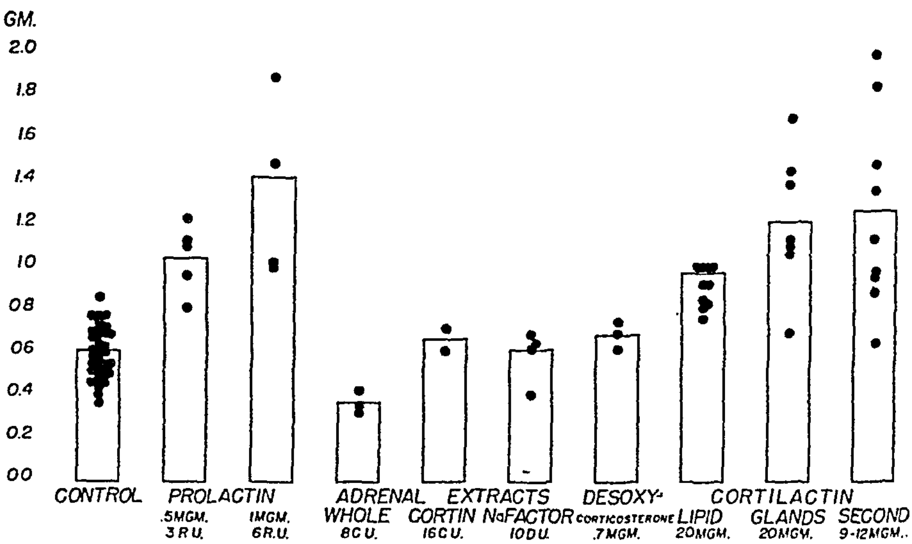

Fig. 1. Pigeon crop gland (dry weight) response to various preparations. The 20 mgm. dose made from glands was from the first iso-electric precipitation. The 9–12 mgm. dose was from the second iso-electric precipitation.

According to the pigeon test the best cortilactin preparation possessed about one-tenth of the potency of purified prolactin.

Rat lactation response. Controls. In order to ascertain the effect of the special diet on the growth of young during lactation, twenty-six normal female rats were placed on the test diet after they had become pregnant. On the day of parturition the number in each litter was reduced as indicated. The weights of the mother and litter were determined daily for 17 days. The average change in the weight of the mother was +8 per cent, ranging from −2 to +23 per cent. All young survived. The average birth weight of the individual pup was 5.3 grams ranging from 4.1 to 6.3 grams. At 17 days the average weight of the individual pup was 29.2 grams ranging from 22.5 to 34.6 grams.

Effect of adrenal preparations. Seven pregnant rats were adrenalec-

tomized just before parturition. They were injected twice daily with enough unfractionated extract (containing 8 c.u. of cortin) to maintain the mother in good condition. Four litters died, while deaths in the other litters reduced the total survival to 31 per cent. The average weight of the surviving pups on the seventeenth day was 17.1 grams.

Four adrenalectomized pregnant rats were treated as above, except that they were given double the first dose of unfractionated extract. The survival of the pups was 76 per cent with an average weight per individual of 20.9 grams at 17 days.

Six adrenalectomized pregnant rats were given treble the first dose of unfractionated extract. In this case the survival of the pups was 87 per cent with an average individual pup weight of 21.2 grams at 17 days.

Adrenal extract is particularly effective in the rat when administered orally (12). Therefore, the following experiment was tried. Three adrenalectomized pregnant rats were injected daily with 8 c.u. of unfractionated extract and in addition were given about 3 c.u. daily of unfractionated extract in their drinking water. Although the survival of the pups was 100 per cent, the average weight of the individual pup at 17 days was 17.3 grams which is far short of normal (fig. 2).

Addition of cortilactin fractions. Nine pregnant females were adrenalectomized and treated with a maintenance dose of unfractionated adrenal extract (8 c.u. of cortin daily). In addition they were injected daily with 10 mgm. of the first iso-electric precipitate (curve "cortilactin I" in fig. 2). The survival of the pups was 88 per cent as compared to 28 per cent in those pups whose mothers received no cortilactin. The average weight of the individual pup at 17 days, however, was only 17.3 grams.

Five adrenalectomized pregnant rats were injected with unfractionated extract containing 8 c.u. of cortin, given 3 c.u. of cortin orally and injected with 1 mgm. of the second iso-electric precipitate daily (curve "cortilactin II" in fig. 2). The survival of pups was 100 per cent while the average weight of the individual pup at 17 days was 28.26 grams ranging from 26.0 to 33.0 grams. This indicates complete replacement therapy.

The average growth curve of surviving pups is shown in figure 2. However as each animal died, one less was included in the average so that a curve from litters with a small percentage survival does not give a true picture of lactation effects.

In those litters where the survival was not 100 per cent, the time and amount of reduction follow: Treatment with unfractionated extract (8 c.u.)—1st day, 81 per cent; 2nd day, 79 per cent; 3rd day, 71 per cent; 5th day, 69 per cent; 8th day, 67 per cent; 10th day, 57 per cent; 11th day 53 per cent; 12th day, 43 per cent; 13th day, 41 per cent; 14th day, 33 per cent; 15th day, 31 per cent. Treatment with unfractionated extract (16 c.u.)—1st day, 96 per cent; 2nd day, 79 per cent; 6th day, 75 per cent.

Treatment with unfractionated extract (24 c.u.)—1st day, 97 per cent; 3rd day, 94 per cent; 4th day, 86 per cent. Treatment with unfractionated extract (8 c.u.) and cortilactin (cortilactin I)—1st day, 93 per cent; 2nd day, 91 per cent; 9th day, 88 per cent.

Increase in the amount of extract over that required to maintain the mother added to the number of pups surviving, but the weight of the individual pup remained low. Oral therapy supplement further increased

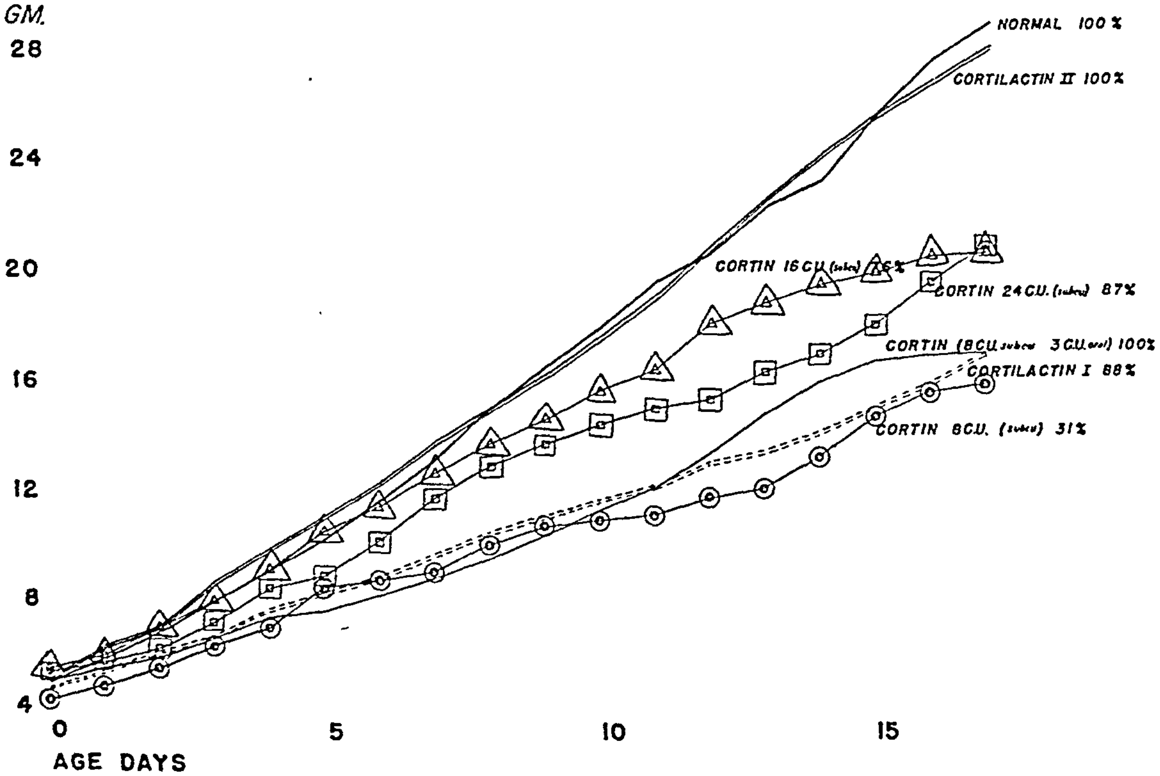

Fig. 2. Growth curves of young rats based on average age of individual pups. All injections subcutaneous except where indicated. The cortin assay is indicated as cat units in the chart. Rats treated with "cortilactin I" were also given daily subcutaneous injections of unfractionated extract containing 8 c.u. of cortin. Rats treated with "cortilactin II" were given subcutaneously, unfractionated extract containing 8 c.u. of cortin and orally, unfractionated extract containing 3 c.u. of cortin.

survival to 100 per cent with no change in pup weight. The addition of the second precipitate (cortilactin II) raised the pup weight to normal.

Effect of cortilactin on gluconeogenesis. Four cortilactin injected rats showed an average of 0:04 per cent liver glycogen, the values ranging from 0.01 to 0.07 per cent. The liver glycogen of the four saline controls averaged 0.16 per cent with a range of 0.06 to 0.30 per cent, while that of the four corticosterone controls averaged 4.18 per cent ranging from 3.55 to 5.96 per cent.

DISCUSSION. Carr's (13) failure to support lactation in adrenalectomized rats by means of adrenal extract prepared by Swingle and Pfiffner's method may have been due to inadequate dosage. Later work of Swingle and Pfiffner (1) showed that their preparation maintained adequate lactation in the dog. Therefore dosage or species difference must have been the explanation.

The "unfractionated adrenal extract" which we used to maintain the adrenalectomized mother rats contained both cortin and sodium factor but little or no cortilactin.

Our cortilactin preparations were made from whole adrenal glands. Therefore whether cortilactin was present in either cortex or medulla or both was not indicated. However the preparations of Brownell, Lockwood and Hartman (2) were made entirely from dissected cortex. Cortex must, therefore, be a source of cortilactin. Our normal animals showed much better survival than did those of Brownell et al. (2). This was due to a better diet and to reduction of the number in the litter to six.

Gaunt and Tobin (14) found that a dosage of extract twice that necessary to maintain adrenalectomized mother rats was adequate for survival and growth of the pups. However they reduced their litters to four instead of six animals.

The iso-electric precipitation method of Riddle, Bates and Dykshorn (5) has yielded lactogenic substances from liver, blood and urine according to Ehrhardt and Voller (15) and Cunningham et al. (16).

The observation that cortilactin will stimulate crop gland formation and that it can be prepared by the same method used for prolactin, raises the question as to whether they are identical. This is answered by Gaunt and Tobin (14) who found that prolactin had no effect on lactation in the adrenalectomized rat.

We have no suggestion to offer as to the mode of action of cortilactin. It apparently has nothing to do with gluconeogenesis.

We wish to thank Dr. Oliver Kamm of Parke, Davis and Company for adrenal glands, Mr. H. W. Rhodehamel of Eli Lilly and Company for prolactin and Dr. R. D. Shaner of Roche-Organon Incorporated for desoxycorticosterone.

SUMMARY

A lactation factor has been prepared from the adrenal by iso-electric precipitation. The pigeon crop gland response was used for its assay. The best cortilactin preparation possessed about one-tenth of the potency of purified prolactin when tested by the crop gland method. The daily injection of 1 mgm. of the cortilactin preparation into an adrenalectomized rat maintained on cortin enabled her to lactate normally. The pup sur-

vival was 100 per cent and their average weight was normal. Cortilactin plays no rôle in gluconeogenesis.

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CHANGES OCCURRING IN THE BLOOD AND TISSUE OF CHICKENS DURING COCCIDIOSIS AND ARTIFICIAL HEMORRHAGE[1]

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Studies on the blood of chickens infected with cecal coccidiosis show a marked increase in the blood sugar. The initial rise is manifest at the beginning of the fifth day at which time the internal hemorrhage due to the disease also begins. As the bleeding becomes more profuse, the blood sugar value continues to rise until the seventh day of infection when the disease has run its course. The loss of blood has been indicated by Pratt (1940) to be responsible for the blood sugar increase during the hemorrhagic phase of the disease. The author (1941), in attempting to replace the deficient constituents of the circulating fluid with concentrated physiological saline, obtained a lower blood sugar level than ordinarily present during coccidiosis. This presented the possibility that the increased blood sugar value during an infection might be in some way related with the chlorides of the blood.

An experiment was undertaken to study the relationship of the sugar and chloride and the changes brought about by coccidiosis: 1, the blood chlorides and sugars were determined before and during the course of an infection; 2, sugar and chloride determinations were made following artificial hemorrhage and these results were compared with those of coccidial bleeding; 3, gum acacia solution was injected in order to replace the blood volume which was lost during artificial bleeding and the resulting chloride values were observed; 4, tissue chloride determinations were obtained from normal and infected animals.

MATERIALS AND METHODS. The experimental animals were Single Comb White Leghorn chickens furnished by the Department of Poultry Husbandry of the University of Wisconsin. The chicks, obtained when one day old, were kept free of infection in sterilized cages and given the feed and care described by Herrick, Ott and Holmes (1936). The chickens were

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infected orally, by means of a pipette, with about 1 cc. of a pure culture of *Eimeria tenella* containing approximately 200,000 viable coccidia oöcysts. The birds were off-feed 19 hours preceding all determinations in order to obtain a basal level from which to measure the changes due to the disease. This procedure was necessary to remove the fluctuations brought about by *ad libitum* feeding.

The blood and tissue chloride determinations were carried out according to the procedure of Van Slyke (1923). The chloride concentrations have been expressed in the terms of NaCl. The blood sugar values were determined colorimetrically by the Folin-Wu method (1920) with an Evelyn photoelectric colorimeter. The blood samples were obtained in all cases by cardiac puncture. The animals were not anesthetized and no anticoagulants were used since the blood was tested as soon as it was drawn. The tissue samples were taken in duplicate and triplicate from the right and left pectoral muscles.

The gum acacia which was used for transfusions was a sodium chloride-free preparation made by Lilly. The gum acacia was made up to a 6 per cent solution by diluting with distilled water. The chloride-free compound was used so that the effect of the transfusion could be judged by the changes in the chloride value. Transfusions were made directly into the heart with a syringe.

EXPERIMENTS. 1. *Blood chloride and sugar changes during cecal coccidiosis.* The animals used in this experiment were divided into two groups; one was infected, the other was not and served as a control. The chloride values of both were obtained before oöcysts were administered and then again on the 4th, 5th, 6th and 7th days following infection. According to the data of table 1, the chickens before infections collectively averaged 472 mgm. of sodium chloride per 100 cc. of blood. On the fourth day the infected group averaged 465 mgm., and on the 5th day was also slightly lower than the preinfection value. A rise was manifested on the 6th day and continued upward to 550 mgm. on the 7th day of the infection. The values of the uninfected controls remained fairly close to the preinfection level of 472 mgm. The chlorides of these animals averaged 465, 467, 454 and 478 mgm. from the 4th through the 7th day respectively. At this time, coccidiosis had effected an average rise of 86 mgm. in two days.

To ascertain whether any relationship existed between this rise in the chlorides and that of the blood sugars during coccidiosis, the two were graphed. The chlorides of the above experiment were compared with the blood sugars of a typical case of coccidiosis. The blood sugar values are from the author's (1941) previous experiment. Before infection the average sugar content of the blood was 175 mgm. per 100 cc. of blood. The values from the 4th through the 7th day show a definite rise as follows: 185, 236, 259 and 251 mgm. per 100 cc., an average increase of 76 mgm.

When the blood chlorides were graphed against the blood sugars, it was apparent that the increase in the chlorides occurred at least a day following that of the sugar. The sugar of the blood showed a definite ascent by the 5th day, reached a peak on the 6th day, and then began to taper off. The chlorides remained fairly close to the control value until the 6th day, when a significant increase was noted, and then continued their rise through the 7th day. It appeared that the mechanism operative in producing the rise allowed for a lag of a day for the chlorides in comparison with that of the sugars.

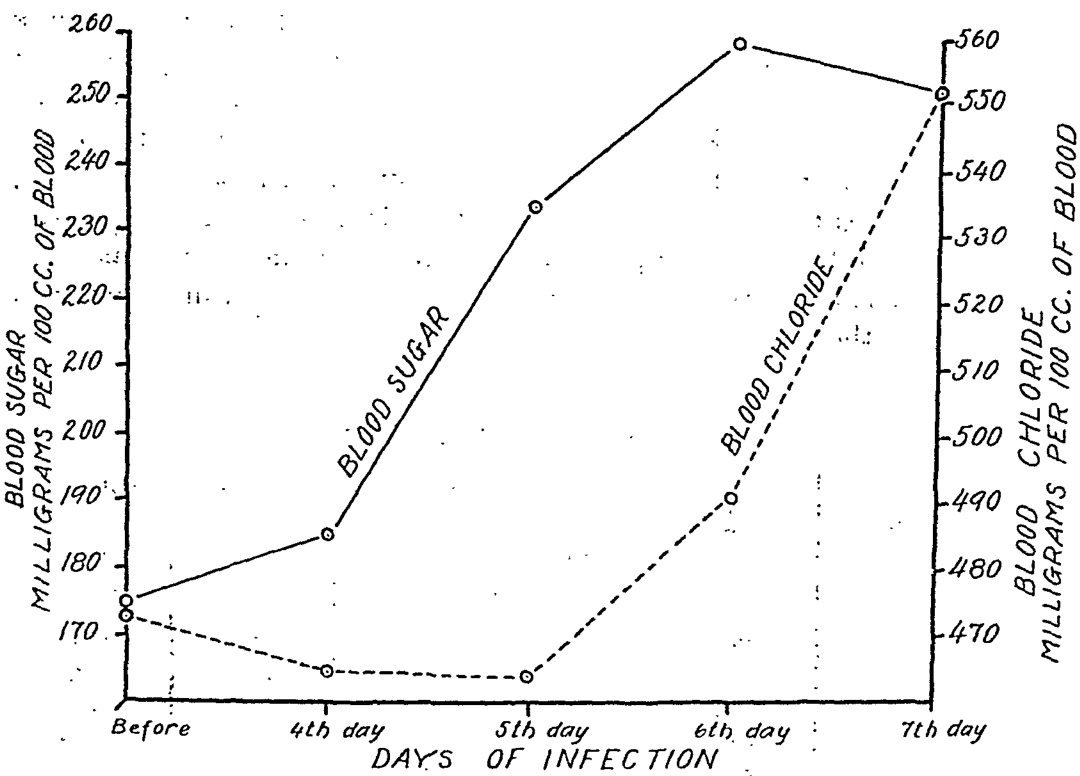
TABLE 1

Effect of coccidia on blood chlorides

CHICKEN NUMBER	BEFORE INFECTION	DAYS AFTER INFECTION (RESULTS IN MGM. OF NaCl PER 100 CC. OF BLOOD)			
		4th	5th	6th	7th
Infected					
1	485	437	454	500	558
2	434	460	449	446	536
3	482	482	467	460	549
4	487	470	464	536	Dead
5	477	474	487	512	556
Uninfected					
6	457	455	447	454	477
7	460	460	469	444	502
8	477	483	482	422	437
9	482	459	469	464	483
10	477	469	467	485	492
Résumé					
Infected	473	465	464	491	550
Uninfected	471	465	467	454	478

2. *Blood chloride and sugar changes following artificial hemorrhage.* The second phase of the experiment concerned the chloride changes that occurred following artificial hemorrhage. By such a procedure Pratt (1940) was able to obtain a rise in the blood sugar similar to that produced by an infection of coccidia. This portion of the work was undertaken to determine whether this type of bleeding would promote a rise in the chlorides in the same way as an infection.

The hemorrhage was produced by withdrawing the blood by direct cardiac puncture. Thirteen chickens of various ages and weights were tested by this procedure. The amount of blood which was taken varied according to the size of the bird and the quantity which could be removed by one puncture without distressing the animal. The average volume that



BLOOD SUGAR AND CHLORIDE CHANGES DURING CECAL COCCIDIOSIS

TABLE 2

Effect of artificial bleeding on blood sugar and chloride

NUMBER	WEIGHT	AMOUNT BLED	BLOOD CHLORIDE		BLOOD SUGAR	
			Before	After	Before	After
	gm.	cc.	mgm. per 100 cc. of blood			
1	1330	39	478	519	180	245
2	1420	37	462	486	162	239
3	1580	38	478	503	181	259
4	1420	42	453	528	173	259
5	1490	32	511	543	195	261
4*	873	23	442	528	199	244
5*	790	25	497	543	198	247
6	1290	30	503	536	168	228
7	740	25	486	557	196	240
8	838	25	478	561	192	246
9	840	15	483	519	207	225
10	884	24	445	536	186	268
11	886	23	453	536	182	260
Average			474	530	186	248

* Tested two months previous.

was withdrawn was 28 cc.; the minimum 15 cc., and the maximum was 42 cc. The chloride content was determined at the time of bleeding and three hours later. The difference between the two was considered as due to the removal of blood. Blood sugar determinations were also made at the same time.

The data from table 2 show that with bleeding the sodium chloride content increased from an average of 474 to 530 mgm. per 100 cc. of blood, an average rise of 56 mgm. This change compared favorably with the 72 mgm. increase obtained with an infection of coccidia (see table 1). At the same time the blood sugar level was in line with that of an infection, as graphed above. The results tended to indicate that the rises were due to the loss of blood in both cases.

An examination of two of the birds (nos. 4 and 5) indicated the constancy of the results which were obtained. These two chickens had been tested two months previously. In regard to no. 4, the chlorides increased at the initial bleeding from 453 to 528 mgm. Two months later, the determinations were 442 mgm. before and 528 mgm. after bleeding. Results of fairly similar uniformity were also obtained for the blood sugars.

3. *Blood chloride level following artificial hemorrhage and gum acacia injection.* It was apparent that the rise in blood chloride and blood sugar brought about by an infection of coccidiosis could be simulated by artificial hemorrhage. To further emphasize that this rise in the chlorides with artificial bleeding and with infection was due to loss of blood, an experiment was set up to replace the deficient volume in artificially bled animals. In a previous experiment, the author had attempted such a procedure by feeding concentrated physiological saline solution to infected animals. This was accomplished when the birds drank excessively of water and showed a lowered blood sugar level. It was thought that this was due to excess water intake and blood volume dilution. In this experiment, the deficient volume was replaced directly by 6 per cent sodium chloride-free gum acacia.

These animals were bled by a single direct cardiac puncture and the blood withdrawn within 10 seconds or less. Immediately thereafter, in some cases without removal of the needle, the gum acacia was injected into the heart. About two-thirds of the volume of blood that had been withdrawn was replaced by the injection of the 6 per cent gum acacia solution. Six chickens were so treated and the results recorded in table 3.

The data indicated that when the gum acacia injections were given after excessive bleeding, no rise in the blood chlorides occurred. No changes were noted after a three hour period, or even after 24 hours had elapsed. The average chloride content of the blood was 481 mgm. before and after the bleeding with gum acacia treatment. The individual differences at the two bleedings were also very close.

4. *Tissue chlorides of infected and normal chickens.* It was apparent

from the previous experiments that there was a rise in the blood chlorides with artificial bleeding as well as with coccidia hemorrhage. There is no single salt depot in the body upon which the chicken may draw to raise this chloride content. The only probable source of chloride which is available is that of the tissue. With this in mind, an experiment was conducted to determine the tissue chlorides of normal and infected animals. The methods for tissue chlorides are known to be unreliable and so deter-

TABLE 3

Six per cent sodium chloride-free gum acacia after mechanical bleeding

NUMBER	WEIGHT	AMOUNT BLED	GUM ACACIA	BLOOD CHLORIDES (RESULTS IN MGM. PER 100 CC. OF BLOOD)		
				Before	After	24 hours later
	gm.	*cc.*	*cc.*			
1	1030	28	18	479	477	477
2	1005	25	16	464	472	464
3	1195	30	20	479	477	472
4	1100	30	20	483	470	470
5	920	18	12	496	495	
6	800	23	16	487	495	
Average				481	481	472

TABLE 4

Effect of coccidiosis on tissue chloride

DAY OF INFECTION	DETERMINATIONS	DAILY AVERAGE	AVERAGE
		mgm. per 100 grams tissue	*mgm.*
Infected			
5	28	46.24	
6	24	47.18	
7	26	46.51	46.62
Uninfected—control			
5	26	49.33	
6	28	51.53	
7	21	51.03	50.62

minations were made in duplicate and triplicate for each animal. The uninfected controls averaged 50.62 mgm. of sodium chloride per 100 grams of tissue. A down-trend was evident in the infected birds on the 5th, 6th and 7th day after infection. The average values for these three days were as follows: 46.24, 47.18 and 46.51 mgm.

Discussion. One of the most notable changes in the blood of chickens following coccidia hemorrhage is the rise in the blood chlorides. An in-

crease of almost the same magnitude is apparent when the chickens are artificially bled. There is at present no obvious evidence of a salt deposit upon which the body can draw to account for the above increased blood chlorides. The drop in the chloride content of the tissue during an infection indicates that the increase in the chlorides of the blood may be derived from the tissue. Starling (1909) stated that after artificial hemorrhage there may be a passage of water and salts from the extra-vascular fluids into the blood vessels.

This influx of salts and water into the blood maintains the circulatory volume. Does this increase in chlorides also help to maintain the osmotic pressure of the blood, which according to preliminary determinations remains relatively constant following both types of hemorrhage? The results of injecting 6 per cent sodium chloride-free gum acacia solution also seems to point in this direction. This solution not only replaces the lost volume but also maintains the osmotic pressure by taking the place of the plasma proteins which were lost by bleeding. Bayliss (1920) indicated this by showing the expediency of injecting gum-saline rather than saline after hemorrhage in an attempt to maintain blood volume and blood pressure. In this experiment there was no rise in the blood chlorides after hemorrhage and gum acacia injection indicating, at least, that when the osmotic pressure of the blood was maintained by some other medium, the chlorides did not rise.

The increase in the chlorides with artificial hemorrhage and with coccidiosis parallels the work of Pratt (1940) with blood sugar. He showed that extensive cardiac hemorrhage artificially produced in normal chickens caused a rise in the blood sugar of the same degree as that caused by coccidiosis. He attributed this hyperglycemia to the muscle and perhaps liver glycogen. To account for the blood chloride increase of this experiment, a decrease in the tissue chloride has been indicated. Although the drop in the tissue chloride was only 4 mgm. per 100 grams of tissue, the amount which was lost by the animal tissues as a whole was quite large and could account for at least a good share of the chloride rise of the blood.

CONCLUSIONS

1. There is an increase of the blood chloride on the 6th and 7th day of an infection of cecal coccidiosis in chickens.
2. The rise in the blood sugar, due to coccidiosis, is apparent on the 5th day, a day prior to the rise of the chlorides.
3. Artificial hemorrhage produces an increase in the blood chloride and sugar approximate to that brought about by bleeding from coccidiosis.
4. The chloride content of the blood is maintained at the normal level after severe artificial hemorrhage by the injection of 6 per cent sodium chloride-free gum acacia solution.

5. The chloride content of the muscle shows a downward trend during coccidiosis and may account in part for the rise in the blood chloride.

Acknowledgments are made to Drs. C. A. Herrick and W. H. McShan for their many helpful suggestions.

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THE EFFECT OF INORGANIC IONS ON GASTRIC SECRETION IN VITRO

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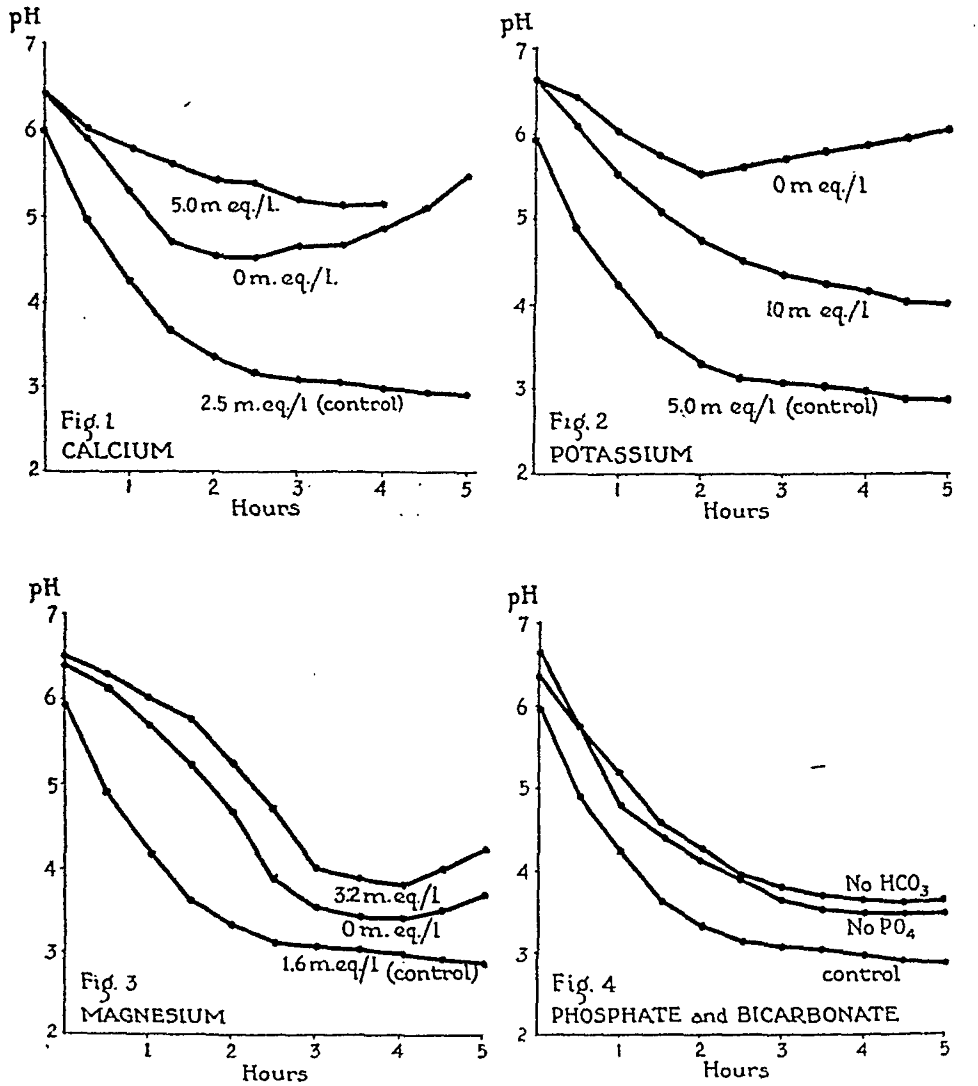
It has been reported that the isolated gastric mucosa of the frog will form acid when properly mounted in a bath consisting of two chambers separated by the gastric mucosa (1, 2). We have recently demonstrated that this *in vitro* production of acid represents a true secretory process on the part of the gastric glands (3). The present investigation is concerned with the response of the isolated glands to changes in their ionic environment. This was undertaken in order *a*, to determine whether glandular tissue resembles smooth muscular tissue in its requirements for optimum concentration of various ions, and *b*, to determine, if possible, which ions might be involved in the process of acid formation in the stomach.

METHODS. The gastric mucosa of fasted frogs was dissected from the muscularis and mounted in the dual-chambered bath exactly as previously described (3). This bath consists of one chamber filled with an isotonic salt solution which receives the secretion, and a second chamber containing a nutrient solution consisting of 71.75 mM./l. of NaCl, 20.0 of Na_2CO_3, 5.0 of KCl, 1.25 of $CaCl_2$, 1.20 of sodium phosphate buffer (pH 7.4), 0.80 of $MgCl_2$. Glucose was added in a concentration of 50 mgm. per cent. This solution was aerated with 5 per cent CO_2 and 95 per cent O_2; the secretory solution was aerated with air, mainly for purposes of stirring.

In order to determine the effect of varying the ionic composition of the nutrient solution, the various ions were successively doubled in concentration and omitted entirely. The altered ion was replaced by or substituted for an equivalent amount of NaCl in order to maintain isotonicity. When the bicarbonate ion was omitted, the nutrient solution was aerated with pure O_2, and the air bubbled through the secretory solution was freed of CO_2.

The changes in the pH of the secretory solution were followed over a period of five hours, using a glass electrode as previously described. The resulting curves were compared with a series of control curves obtained without alteration of the nutrient solution.

RESULTS. The results of the various experiments are presented in the



Figs. 1–4. The effect of alterations of ion concentration on the formation of acid by the isolated gastric mucosa of the frog.

TABLE 1

Average, maximum, and minimum low pH values

	CONTROL	Ca		K		Mg		PO_4 OMITTED	HCO_3 OMITTED
		Omitted	Doubled	Omitted	Doubled	Omitted	Doubled		
Number of trials	11	8	8	8	6	4	4	8	12
Average	2.89	4.51	4.90	5.49	3.83	3.38	3.68	3.43	3.48
Maximum	3.22	6.42	5.72	6.50	5.01	3.52	4.07	4.60	4.58
Minimum	2.54	3.39	3.71	3.51	3.07	3.21	3.21	2.87	1.49

figures and the table. In the former, the average pH curves over the five hour period are shown for each type of experiment; in the latter, the average, together with the maximal and minimal values for the lowest pH attained in the individual trials for each type of experiment are shown.

In the eleven control experiments the curve reached an average low level of pH 2.9 at the end of five hours. In the individual trials the low point ranged from 3.22 to 2.54. The degree of uniformity that is encountered in this type of experiment is indicated by the close similarity between these eleven entirely new and the eight previously published control curves (3).

When the calcium ion was entirely omitted from the nutrient solution, the pH fell more slowly, did not fall as far, and began to rise again before the conclusion of the five hour period. A similar, but more marked, interference with acid formation occurred when the calcium concentration was doubled.

In the case of the K ion, its omission seriously interfered with secretion, whereas doubling its concentration produced less extensive inhibition. Alteration in either direction of the Mg ion concentration produced mild inhibitory effects which consisted mainly of delaying the onset of secretion.

The ions whose absence appeared to have the least injurious action were found to be PO_4 and HCO_3, the two ions which are generally considered to be involved in the formation of HCl by the gastric glands. The lowest pH which has so far been recorded, namely, 1.49, occurred in the absence of the HCO_3 ion.

Discussion. Variations in the ionic composition of the artificial bath affect the *in vitro* secretion of acid by the gastric glands of the frog. In this respect the mucosa is most sensitive to variations in Ca ion, less so to K ion, still less to Mg ion, and scarcely at all to PO_4 and HCO_3 ions. With the single exception of the HCO_3 ion, this series is identical with that demonstrated by Van Dyke and Hastings (4) to hold for uterine smooth muscle *in vitro*. Delrue (2) who employed the isolated gastric mucosa of the frog reported the following series, Ca, HCO_3, K and PO_4. His experiments, however, were performed in the late fall, so that the pH of the secretory solution rarely fell below 5.0. The primary importance of the calcium ion for gastric secretion is also indicated by reports that gastric secretion *in vivo* is inhibited by alteration in either direction of its concentration in the blood (5, 6, 7, 8, 9, 10).

At first glance, the failure of the absence of PO_4 ions to interfere with secretion *in vitro* appears to be contrary to Maly's hypothesis concerning the formation of HCl. However, since the PO_4 ion is not used up in Maly's reactions, a normal initial supply may serve the requirements of

the cell for this ion. Accordingly, the present observations neither support nor deny Maly's hypothesis.

The finding that the omission of the HCO_3 ion does not interfere with the *in vitro* formation of acid is in contradiction to the generally accepted view. There have been a number of reports to the effect that a reduction in blood bicarbonate inhibits gastric secretion (11, 12, 13, 14). In most cases this reduction has been accomplished by hyperventilation, which can reduce the ionic calcium of the blood to the point of tetany. Furthermore, the inhibition of secretion is difficult to demonstrate against a stimulus as potent as histamine (14). The use of the isolated mucosa makes it possible to distinguish between direct and indirect effects of various procedures on the gastric glands, and in this case it would appear that the effects of reduction of the blood bicarbonate are expressed indirectly, since they do not occur *in vitro*.

It would seem unlikely that a cell which requires considerable energy to form its secretory product should be dependent upon an *external* source of CO_2 or bicarbonate, for its own metabolism must supply large amounts of this substance. It has recently been demonstrated that the mammalian parietal cell contains the enzyme, carbonic anhydrase, in greater concentration than the red cell (15, 16). It is probably the function of the enzyme to convert carbon dioxide gas, formed within the cell in large quantities, into ionizable carbonic acid, of which the H ion becomes available for the formation of acid, and the HCO_3 ion for release to the blood stream in exchange for Cl ions. Accordingly, there seems to be no *a priori* reason for believing the acid forming cells to be dependent upon an outside source of CO_2.

CONCLUSIONS

1. The gastric glands of the frog *in vitro* resemble smooth muscle in their sensitivity to alteration in their ionic environment. The descending order of sensitivity to the various ions is as follows: Ca, K, Mg, PO_4 and HCO_3.

2. An *external* supply of PO_4 and HCO_3 ions is not necessary for the formation of acid by the gastric glands of the frog *in vitro*.

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GASTROINTESTINAL TRACT MOTILITY IN THE ABSENCE OF BILE

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A knowledge of the laxative effect of bile was part of the medical lore of the ancient world. The modern contributions to this subject have recently been reviewed by Haney, Roley and Cole (1), who conclude as a result of their own studies on dogs with Thiry-Vella loops that the introduction of bile stimulates the motor activity of the small intestine and that this effect is due entirely to the presence of bile salts. They further suggest that "bile salts may play an important rôle in the normal regulation of the propulsive movements of the small intestine."

While the evidence presented by Haney et al. that bile salts applied to the mucosa of isolated loops will increase loop motility can hardly be questioned, the rôle of the normal biliary secretion in the activation of intestinal motility seemed to deserve further study. Peters (2) has shown that bile salts, in the concentration in which they occur in bile, inhibit absorption from the ileal loops and that this concentration is not normally present in the ileum. That the ileum may be in general less resistant to irritation than the upper small bowel is also indicated by the observations of Dennis (3). Haney et al. do not state the origin of the loops used by them, but if they were segments of ileum one may question the propriety of drawing inferences concerning normal digestive tract behavior from the effects of undiluted bile on such loops.

We have carried out roentgenologic studies in bile fistula dogs using meals containing large and small proportions of fat in order to determine under more physiological conditions the relationship of bile to gastrointestinal motility.

METHODS. Bile fistulae of the "internal" type were made in 8 dogs, using the technique of Kapsinow, Engle and Harvey (4) (anastomosis of gall bladder to right renal pelvis with ligation of the common duct). The animals were maintained on a diet consisting of meat scrap 8, corn meal 30, soy bean meal 50, wheat germ 10, alfalfa leaf meal 1.5, and salt 0.5; the fat content of this diet is approximately 5 per cent. They received parenteral injections of vitamins A, D, E and K since it is known that in

the absence of bile the absorption of the last three of these and of carotene is inadequate.

Two types of barium meals were used. No. 1, a mixed meal, was composed of 100 grams barium sulfate, 100 grams ground beef, and 100 ml. milk. No. 2 (fat meal) consisted of 30 grams barium sulfate, 100 ml. Wesson oil and 100 ml. water. Meal no. 1 was fed to 8 normal and 7 bile fistula dogs, and x-ray films were made 4 and 8 hours after feeding. Meal no. 2 was given by stomach tube to 5 normal and 7 bile fistula dogs, films being made at $4\frac{1}{2}$ and $7\frac{1}{2}$ hours.

TABLE 1

Proportions of meal no. 1 present in various parts of gastrointestinal tract 4 and 8 hours after feeding

NORMAL DOGS				BILE FISTULA DOGS				
Dog number	Stomach	Small intestine	Large intestine	Dog number	Time since operation	Stomach	Small intestine	Large intestine
				4 hours				
1	3/6	3/6		1B	2 weeks	3/6	1/6	2/6
2	3/6	3/6		2B	6 weeks	2/6	3/6	1/6
3	4/6	2/6		3B	3 months	2/6	4/6	
4	4/6	2/6		4B	11 months	3/6	3/6	
5	3/6	3/6		5B	4 months	2/6	2/6	2/6
6	3/6	3/6		6B	4 months	2/6	3/6	1/6
7	3/6	3/6						
				8 hours				
1	2/6	2/6	2/6	1B		tr	2/6	4/6
2	2/6	1/6	3/6	2B			2/6	4/6
3	1/6	2/6	3/6	3B		1/6	2/6	3/6
4	2/6	1/6	3/6	4B		1/6	2/6	3/6
5	1/6	1/6	3/6(E)	5B		1/6	2/6	3/6
6	1/6	1/6	4/6	6B		1/6	2/6	3/6
7	1/6	2/6	3/6					

The dogs used as controls had in every instance been kept in the laboratory on our stock diet for several weeks.

RESULTS. In order to compare the progress of the barium meal in the various animals, the proportions of the meal present in stomach, small intestine, and large intestine were estimated by inspection of the x-ray films. For convenience, the larger amount of barium in meal no. 1 was expressed in sixths, and that in the smaller meal in fourths. The results are given in tables 1 and 2.

We have regarded differences between the normal and bile fistula animals as being significant only when at least 50 per cent of the bile fistula

dogs showed rates of barium movement outside of the limits of variation found in the normal series. On this basis it may be concluded that after a large meal (no. 1) the stomach of the bile fistula dog empties more rapidly and that there is earlier entry of barium into the colon (4 hr. films). There is also evidence in the 8 hour films of more rapid gastric emptying in the bile fistula dogs, but the change from the normal is not definitely significant.

The $4\frac{1}{2}$ hour films taken after the administration of the fat meal (no. 2) show no indubitably significant difference between normal and operated

TABLE 2

Proportions of fat meal (meal no. 2) remaining in various parts of gastrointestinal tract $4\frac{1}{2}$ and $7\frac{1}{2}$ hours after feeding

NORMAL DOGS				BILE FISTULA DOGS						
				MEAL ALONE				MEAL PLUS 3 GRAMS BILE SALTS		
Dog number	Stomach	Small intestine	Large intestine	Dog number	Stomach	Small intestine	Large intestine	Stomach	Small intestine	Large intestine
$4\frac{1}{2}$ hours										
8	1/4	3/4		1B		1/4	3/4	1/4	1/4	2/4
9	2/4	1/4	1/4	2B	4/4	tr		1/4	2/4	1/4
10	tr	4/4		3B	1/4	2/4	1/4	1/4	3/4	
11	2/4	2/4		4B	3/4	1/4		2/4	2/4	
12	2/4	2/4		5B	2/4	2/4		1/4	1/4	1/4(E)
				6B	2/4	2/4		1/4	3/4	
$7\frac{1}{2}$ hours										
8	tr	2/4	2/4	1B		tr	4/4		tr	4/4
9		1/4	3/4	2B	2/4	1/4	1/4	1/4	tr	3/4
10		2/4	2/4	3B		1/4	3/4		2/4	2/4
11	tr	2/4	1/4(E)	4B	3/4	1/4		1/4	2/4	1/4
12	1/4	3/4	tr	5B	1/4	2/4	1/4	1/4	2/4	1/4
				6B	2/4	2/4	tr	tr	2/4	2/4

animals although the barium tends to remain longer in the stomach and to pass more rapidly through the small intestine in the latter. But in the $7\frac{1}{2}$ hour films it is apparent that gastric emptying is definitely slower in the bile fistula dogs. The addition of 3 grams of bile salts to the fat meal increased gastric emptying in the bile fistula dogs so that it fell within normal limits.

It should be noted that the small intestine of the bile fistula dogs contained, on the average, less barium than did that of the normal animals in every series of films except those taken 8 hours after meal no. 1. This

constant difference may indicate a greater irritability of the small bowel in the operated animals.

It has been impossible to correlate the observed changes in motility with post-operative time or with the general condition of the animals. One bile fistula dog was omitted from the series because roentgenologic observations indicated the presence of adhesions about the duodenum which accounted for a greatly increased gastric emptying time in this animal.

Discussion. The increased rate of gastric emptying of the bile fistula dogs after a mixed meal was unexpected and is not easily explained. Fauley and Ivy (3) observed a similar decreased stomach emptying time after ligation of the pancreatic ducts or extirpation of the pancreas; they suggest that the increased appetite of their animals may be responsible, since it is known that the presence of hunger augments gastric evacuation, but point out that unknown factors may be concerned. Some of our bile fistula dogs ate well but others did not, and we can find no indication of any correlation between gastric evacuation and appetite in our series.

The delayed gastric emptying after a fat meal in the bile fistula dog may be more readily accounted for on the basis of known mechanisms. It seems probable that delayed absorption of fatty acids due to the absence of bile would lead to an increased formation of enterogastrone and a greater inhibition of gastric motility. Decreased motility after the fat meal is exhibited only by the stomach and not by the small intestine. The restoration to normal rates by the administration of bile salts is to be expected on this basis.

In general our data lend no support to the concept that bile salts are an important regulator of small intestinal activity. The increased motility produced by the contact of whole bile with isolated intestinal loops (1) is probably attributable to chemical irritation from concentrations of bile salts exceeding those present in the bowel under normal conditions.

It is interesting to speculate on the possible relationship of the increased rate of gastric emptying in the bile fistula dogs to ulcer formation. Some 15 per cent of these animals are known to develop peptic ulcer (6). In view of the known relationship of mechanical factors to ulcer (7), does an increased gastric motor drive secondary to an absence of bile from the intestine contribute to ulcer development?

CONCLUSIONS

1. Bile fistula dogs given a mixed meal exhibit an increase in rate of gastric evacuation and of entrance of barium into the large intestine.

2. After a fat meal, the rate of gastric emptying in the bile fistula preparation is slower than in normal dogs, possibly because of the formation of increased amounts of enterogastrone.

3. The administration of bile salts with the fat meal brings the gastric emptying time of the bile fistula dog to within normal limits.

4. The suggestion that bile salts are an important factor in the regulation of small intestine motility is not supported by our observations.

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FURTHER EVIDENCE FOR THE FLUID CIRCUIT THEORY

THE RATE OF CHLORIDE ACCUMULATION IN THE LOWER ILEUM UNDER CONDITIONS SUITABLE FOR ACTIVE CHLORIDE ABSORPTION

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In an attempt to explain the absorption of chloride from the lower ileum which occurs against concentration gradients, Ingraham, Peters and Visscher (1) proposed the "fluid circuit" theory, which assumes that the absorption of water carries chloride out of the intestinal contents without changing its concentration from that in the lumen while a fluid free of chloride moves from the blood into the intestinal lumen. The theory has been supported by many experiments (1, 2). Most of these, however, would lose their significance if the passage of considerable amounts of chloride from blood to lumen could be demonstrated under similar conditions. The author has therefore studied the accumulation of chloride in initially chloride free solutions in the lower ileum under conditions duplicating as closely as possible those of the previous experiments.

METHODS. Dogs were anesthetized with sodium amytal injected intraperitoneally, 65 mgm. per kgm. In experiments 5 and 6 this was supplemented with small amounts of ether. A loop of lower ileum about 20 inches long and about 6 inches from the cecum was isolated. A rubber cannula was inserted into one end of the loop and a glass cannula in the other. It was then rinsed out with 3 to 4 liters of isotonic NaCl at about 37° until the washings were clear. A rubber plug, having a longitudinal hole nearly but not quite through it for insertion of a needle, was then substituted for the glass cannula, and the loop was again rinsed out to remove traces of blood. After the rubber cannula had been closed with a short length of glass rod, the loop was returned to the abdominal cavity and allowed to rest for 30 minutes. The dog was kept warm with an electric lamp during the entire experiment.

The solutions used are described in the legend for figure 1. In 6 out of 8 experiments they are the same as the solution of $\frac{1}{2}$ isotonic Na_2SO_4 and $\frac{1}{2}$ isotonic NaCl used formerly (2) except that all or nearly all of the NaCl has been replaced by glucose, which was chosen because it is non-irritating and easily absorbed. At the end of the rest period 50 cc. of the

experimental solution at 37° was injected from a syringe into the loop through the rubber cannula. A hypodermic syringe and needle were used at intervals to puncture the rubber plug and withdraw 2 cc. samples. The intestinal contents were gently mixed just before sampling. During all procedures an effort was made to avoid mechanical stimulation of the intestine. Chloride concentrations were determined by the method of Van Slyke (3).

Results. Chloride concentration is plotted against time in figure 1. In no case was a concentration greater than 0.032 per cent NaCl observed. Water absorption was satisfactory in all experiments and averaged 28 cc. per hour. Active chloride absorption occurred at some time during the experiment whenever sulfate was present. In the other experiments active chloride absorption was not demonstrated.

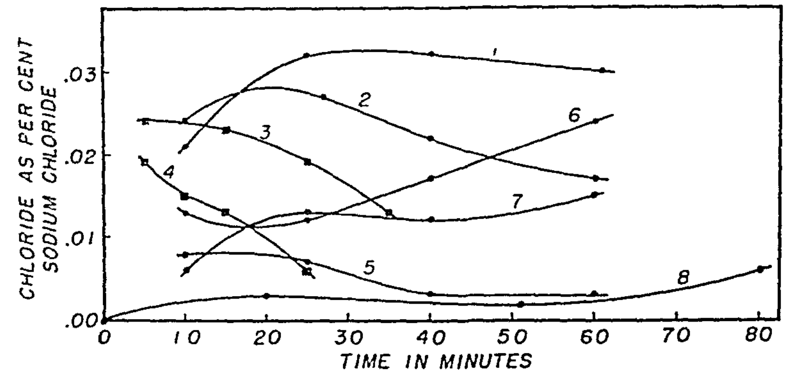

Fig. 1. Accumulation of chloride in the lower ileum. The isotonic solutions used were as follows: for experiments 1, 2, 5 and 8, ½ isotonic Na_2SO_4 and ½ isotonic glucose; for experiments 3 and 4, 44/90 isotonic Na_2SO_4, 44/90 isotonic glucose, and 1/45 isotonic NaCl; for experiments 6 and 7, isotonic glucose.

Discussion. Chloride accumulation in the lower ileum has been previously studied under different conditions. Cohnheim (4) obtained values ranging from 0.035 to 0.22 per cent NaCl. Frey (5, 6) found concentrations of from 0.03 to 0.09 per cent NaCl. In a single experiment Burns and Visscher (7) obtained low values, but chloride concentrations during the first hour were not determined.

The definitely lower rate of chloride accumulation reported here seems to be the result of the special conditions chosen. These include as the most important factors an isotonic non-irritating solution, a 30 minute rest period, minimal mechanical stimulation, and optimal anesthesia. Cobet (8) and Dennis and Visscher (9) have shown that anesthesia promotes active chloride absorption, at least in some dogs.

The present studies fail to reveal any facts which would invalidate or weaken previous evidence for the fluid circuit theory. Therefore reasonable objection to the theory on the basis of accumulation experiments

in the literature is no longer possible. However, the experiments reported here do not constitute actual proof that considerable amounts of chloride do not pass from the blood into the intestinal lumen. It is conceivable that rapid absorption might prevent accumulation in spite of such transfer.

The author (10) has recently developed a general fluid circuit theory which includes diffusion and secretion of chloride and osmosis. Although the differential equation arrived at is rather complicated, some simplification has resulted from the use of the variable, C_e, the effective concentration of chloride in the fluid passing into the intestinal lumen. According to the theory, if the slope of the concentration-time curve, dc/dt, is negative, C_e is less than the concentration in the loop at the time. Examination of figure 1 will demonstrate that wherever this principle can be applied, C_e is less than 0.033 per cent NaCl. If we consider the earliest negative slopes, we find an average upper limit for C_e of 0.018 per cent NaCl. The use of the original simple form of the fluid circuit theory in the earlier experiments therefore appears to be justified. The general fluid circuit theory should be applied when movement of chloride into the intestinal lumen or osmosis is not negligible.

SUMMARY

Under the conditions of these experiments chloride accumulates very slowly in originally chloride free solutions in the lower ileum. The highest concentration observed was 0.032 per cent NaCl.

The average upper limit of the effective chloride concentration of the fluid entering the intestinal lumen was 0.018 per cent NaCl according to the general fluid circuit theory.

The results support previous experiments on the fluid circuit theory.

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THE RELATION BETWEEN THE PHOSPHATE CHANGES IN BLOOD AND MUSCLE, FOLLOWING DEXTROSE, INSULIN AND EPINEPHRIN ADMINISTRATION[1]

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It has been well established that the administration of either dextrose, insulin or epinephrin to normal animals causes a decrease in the inorganic phosphate of the serum or whole blood (1, 2, 3, 4). It has also been shown that there is an increase in the hexose monophosphate content of skeletal muscle after insulin and epinephrin injections (1, 5). Since the concept was first advanced by Cori (1), it has been generally assumed that the inorganic phosphate which leaves the blood under the above circumstances enters the muscle together with blood glucose to form the muscle hexose monophosphate which appears. The fact that insulin which lowers the blood sugar and epinephrin which raises it both cause a fall in blood inorganic phosphate has been explained on the basis of experiments on adrenalectomized animals. In the latter, Cori found that insulin did not decrease the muscle hexose monophosphate while epinephrin still produced its usual effect. He concluded that when insulin acts on the blood and muscle phosphates in the normal animal, it does so by causing a reflex epinephrin secretion consequent to the hypoglycemia which the insulin induces.

Several authors have noted serious and hitherto unexplained objections to the above concepts:

1. The administration of sugar causes a fall in blood inorganic phosphate, but does not change the hexose monophosphate level in the muscle (1, 2).

2. The administration of sufficient sugar simultaneously with insulin to avoid the reflex secretion of epinephrin due to hypoglycemia results in a fall in blood inorganic phosphate without a change in the muscle hexose monophosphate (1, 2).

3. In adrenalectomized animals, in which Cori (5) found that insulin did not increase muscle hexose monophosphate, Ellsworth and Wein-

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stein (6) nevertheless reported that the serum inorganic phosphate was lowered to the same degree as in normal animals.

4. Pollack et al. (7, 8), who demonstrated that glucose and insulin caused a fall in the serum inorganic phosphate of blood perfused through the isolated limbs of dogs, failed to discover any change in the hexose monophosphate content of the perfused muscles.

5. In the perfused hind limbs of cats Lundsgaard (9) found that the active deposition of glycogen in the muscles following insulin addition, was accompanied by a fall in the inorganic phosphate of the perfusing blood. However, when glycogen deposition was accomplished by raising the dextrose level without the addition of insulin, no change in the blood inorganic phosphate occurred.

The present work accounts for the apparent inconsistencies noted above, and offers a satisfactory explanation for the phosphate changes in blood and muscle which follow the administration of dextrose, insulin and epinephrin.

METHODS. The present work was done on normal, depancreatized and adrenalectomized dogs. The experiments with adrenalectomized animals were done under nembutal anesthesia, the actual experiment following within an hour after the removal of the adrenals. The purpose of this procedure was to obtain a preparation in which no reflex secretion of epinephrin was possible but which did not suffer from adrenal cortical insufficiency. Preliminary control experiments had shown that the anesthetic used did not interfere with any of the methods of producing phosphate change in normal animals.

The amount of glucose administered throughout these experiments was 1.75 gram/kgm. body weight in a 30 per cent solution by vein. The dosage of insulin varied between 0.3 and 1 unit per kgm. body weight given subcutaneously. The dose of epinephrin was 0.1 mgm. per kgm. body weight in a 1:2000 solution, subcutaneously.

Inorganic and total acid soluble phosphates were determined by the method of Fiske and Subbarow adapted to the photoelectric colorimeter (10). Muscle hexose monophosphate was determined by the method of Cori and Cori (11). Blood sugar estimations were made by the Somogyi modification of the Shaffer-Hartman method.

RESULTS. Table 1 summarizes our data on the changes in blood inorganic phosphate and total acid soluble phosphate following the administration of glucose, insulin and epinephrin respectively to 75 dogs. It may be seen that whatever the substance administered and regardless of the type of animal used, the fall in inorganic phosphate was not accompanied by a fall in total acid soluble phosphate of the blood. The latter actually rose in most cases for reasons which we cannot explain. In a number of experiments the determination of cell volume by the hemato-

crit method failed to account for the change in total acid soluble phosphate. Under these circumstances it is evident that one cannot explain the fall in inorganic phosphate by supposing that it leaves the blood to enter the muscle, since that would necessitate a fall in total phosphate. The possibility that the inorganic phosphate actually goes into the muscle but that an equivalent or greater amount of organic phosphate enters the blood simultaneously, is discounted by such work as that of Pollack et al. (7). He found no change in the phosphate partition of perfused muscle, following the fall in inorganic phosphate in the perfusing blood. It must be concluded that an esterification of the inorganic phosphate occurred within the blood in Pollack's experiments, and presumably in ours. However, it is impossible to exclude at the present time, the possibility of phosphate transfers between the blood and organs other than muscle. The crucial proof for a phosphate esterification within the red blood cell under the influence of insulin, would be the demonstration of such an effect on whole blood in vitro. A number of attempts in this direction have thus far met with no success.

TABLE 1

Milligrams per cent change in blood inorganic phosphate (P_0) and in total acid soluble phosphate (P_T)

The maximum decrease in blood inorganic phosphate (P_0) obtained with glucose in any depancreatized animal was 0.4 mgm. per cent. Hence no change in P_0 of this amount or less was considered to be significant throughout our work.

TYPE OF ANIMAL	GLUCOSE					INSULIN					EPINEPHRIN				
	Number of dogs	Decrease in P_0			Rise in P_T	Number of dogs	Decrease in P_0			Rise in P_T	Number of dogs	Decrease in P_0			Rise in P_T
		Min.	Max.	Av.	Av.		Min.	Max.	Av.	Av.		Min.	Max.	Av.	Av.
Normal	5	0.5	1.2	0.8	2.0	20	0.7	2.0	1.2	2.0	9	0.4	1.7	1.1	2.0
Depancreatized	7	0	0.4	0.2	1.0	9	1.3	2.8	1.9	1.0	14	0.6	0	0.3	3.0
Adrenalectomized	3	0.7	2.8	1.6	6.0	7	0.5	2.0	1.2	3.0	3	1.1	1.2	1.2	3.0

Further inspection of our data in table 1 also shows that insulin caused a significant fall in inorganic phosphate in all three types of animals, while glucose and epinephrin were effective in the normal and adrenalectomized animals but not in the depancreatized ones. It is apparent that the fall in inorganic phosphate occurs only in the presence of the pancreas or of administered insulin. Since neither endogenous nor administered epinephrin need be present for the effect it may be supposed that the fall in inorganic phosphate which follows the injection of epinephrin into normal animals is a result either of a rise in the blood sugar level, which

would be equivalent to sugar administration, or to a reflex secretion of insulin consequent to the hyperglycemia.

The fact that the change in blood phosphate following glucose, insulin or epinephrin administration is due to a transformation of the inorganic phosphate to an organic form outside the muscle, leaves unexplained the previously observed simultaneous change in muscle hexose monophosphate following insulin or epinephrin administration, and the absence of this effect when glucose is given. Table 2 summarizes simultaneous observations on blood phosphate and muscle hexose monophosphate, and makes it possible to correlate the blood and muscle effects in a satisfactory manner. It may be seen that in the absence of the adrenal glands insulin did not cause a rise in muscle hexose monophosphate although it produced its

TABLE 2

Change in inorganic phosphate (P_0) and total acid soluble phosphate (P_T) of the blood, and in hexose monophosphate (HmP) of the muscle

EXPERIMENTAL CONDITIONS	DOG NUMBER	CHANGE IN BLOOD		CHANGE IN MUSCLE
		P_0	P_T	HmP*
		mgm. per cent	*mgm. per cent*	*mgm. per cent*
Depancreatized	1	−0.3	0	+9.5
Given epinephrin	2	−0.1	+3.0	+10.9
(0.1 mgm. per kgm. subcutaneous)	3	−0.4	0	+9.4
Adrenalectomized	1	−1.9	0	−0.5
Given insulin	2	−1.6	+2.0	−0.3
(0.3 unit per kgm. subcutaneous)	3	−1.2	+3.0	+0.3

* In terms of P.

usual diminution in blood inorganic phosphate. However epinephrin in the depancreatized dog, while causing no significant fall in blood inorganic phosphate, resulted in a definite increase in the muscle hexose monophosphate. It is evident that the rise in hexose monophosphate is a result of epinephrin activity within the muscle, according to its well known action in causing a breakdown of glycogen, as demonstrated in vitro by Cori (12).

It may also be of interest to record certain incidental findings in the course of this work.

a. The presence of the hypophysis is not necessary for the effects on blood phosphate. Hypophysectomized dogs exhibited significant falls in blood inorganic phosphate after dextrose, insulin and epinephrin administration respectively. The "Houssay" animal behaved like the depancreatized dog.

b. The recovery from the fall in blood inorganic phosphate caused by

any of the three agents employed, often continued to levels significantly above the initial control values. Indeed this overshooting was sometimes greater in extent than the initial decrease. This was not accompanied by a comparable increase in the total acid soluble phosphate of the blood.

c. Protamine insulin differed from regular insulin in that the parallelism between the blood sugar and phosphate curves was not maintained with the former. Figure 1 exemplifies this difference.

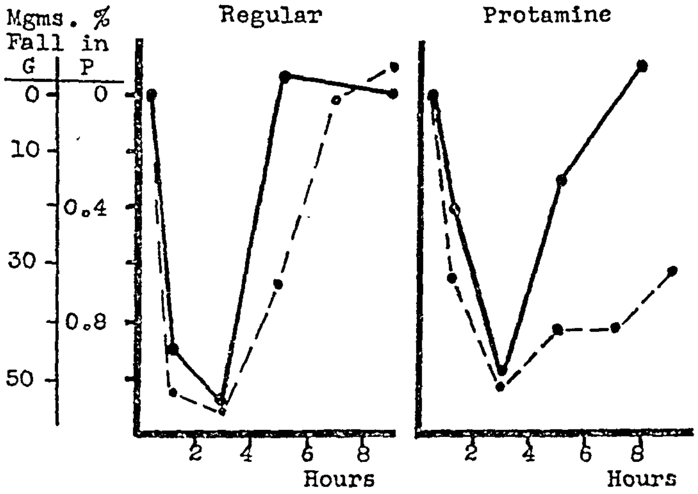

Fig. 1. Illustrating the similarity between the inorganic phosphate curves (heavy continuous lines) in normal dogs, after regular and protamine insulin respectively, despite the difference in the blood sugar curves (broken lines).

Discussion. Our results have reconciled the apparently contradictory previous evidence concerning the changes in blood and muscle phosphate following the administration of insulin, dextrose or epinephrin. It is clear that the confusion has been due to the counter-regulatory actions of the endocrine glands through which excessive insulin activity evokes a secretion of epinephrin and vice versa. When these actions are isolated by excision of the counter-regulating gland the unopposed action of the administered hormone can be observed.

In the normal intact animal epinephrin causes both a fall in the inorganic phosphate of the blood and a rise in the hexose monophosphate of the muscle. It is clear from our work that the change in the blood is not the direct result of epinephrin but is secondary to a reflex insulin secretion. In the absence of the pancreas the change in blood phosphate no longer occurs, although the muscle effect persists. Epinephrin acts directly on the muscle by stimulating the breakdown of glycogen to hexose monophosphate (12).

Insulin also causes both blood and muscle phosphate effects when administered to the normal intact animal. In this case, however, the action

of insulin on blood phosphate is direct because it occurs in the absence of the adrenal glands. The muscle effect of insulin is indirect. It does not occur in the adrenalectomized animal. The responsibility of reflexly secreted epinephrin for the muscle phosphate changes after insulin administration accounts for the absence of these changes when sufficient dextrose to prevent hypoglycemia is administered with the insulin.

The esterification of the blood inorganic phosphate by insulin must be considered in relation to the wider problem of insulin action on carbohydrate metabolism. It seems reasonable to assume that this hormone would have similar effects in tissues other than the blood, although the local situation in which it acts would be expected to alter the manner in which its action would become manifest. This conception is consistent with our previous conclusions as regards the action of insulin in muscle and liver (13, 14), to the effect that insulin catalyzes a phosphorylation which facilitates the entry of blood glucose into the cell or into a metabolic cycle preceding and necessary for both glycogen storage and oxidative breakdown.

CONCLUSIONS

1. Observations on the action of dextrose, insulin and epinephrin respectively on the blood and muscle phosphates of normal, depancreatized and adrenalectomized dogs, indicate that the phosphate changes in blood and muscle are not directly related to each other.
2. The fall in blood inorganic phosphate is due to insulin. It is not reflected in a change in the total phosphate content of the blood, and is probably due to an esterification of the inorganic phosphate outside the muscle.
3. The rise in the hexose monophosphate content of the muscle is due to epinephrin, and results from the breakdown of muscle glycogen.
4. The usual observation of phosphate changes in both blood and muscle after the administration of either insulin or epinephrin to the intact normal animal is due to the reflex evocation of the secretion of one gland by the effects of the hormone of the other gland.
5. The action of insulin in esterifying blood inorganic phosphate, is considered in relation to the general action of insulin on carbohydrate metabolism.

We wish to acknowledge the contributions to this work of Drs. M. D. Allweiss, H. Falk and L. Linn, in the several years during which this paper slowly evolved.

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HEPARIN AND NATURAL ANTIPROTHROMBIN IN RELATION TO ACTIVATION AND "ASSAY" OF PROTHROMBIN

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Clotting-time (C.T.) can be a true measure of thrombin (and prothrombin) only when standardization of the test conditions includes the most rigid control of all inhibitory processes. In view of the growing importance of clinical "prothrombin" tests (15, 16) several recent publications from this laboratory (5, 7, 10) have sought to extend the experimental basis for a revised outlook on clot-inhibitory mechanisms. The present communication deals with the rôle of *antiprothrombins* (13), their occurrence in various clotting systems (including plasma), and the extent to which they may interfere with the interpretation of "prothrombin" assays.

Prothrombin assay technic. Since we are concerned particularly with the isolated first phase of clotting, the method of Quick (15) is not immediately under discussion. Our technic rather resembles the two-stage method of Warner, Brinkhous and Smith (16) except that we are chiefly interested in the underlying principles, development of which goes back to the *thrombin* assays of Fischer (4) and of Eagle (3). The essential feature of the present study is the use of a reference series of thrombin dilutions prepared in each case from the particular prothrombin being studied. This affords a means of controlling both known and doubtful variables and ensures that the clotting-time variations do have the quantitative significance attributed to them upon analysis of the experimental data.

Reagents. Routine throughout these investigations are the use of physiological[1] *saline* (0.9 per cent NaCl) for all solutions and dilutions, and adjustment of the *pH* of all reagents to 7.5 with the aid of dilute acid (N/10 acetic) or alkali (N/10 NaOH) and the glass electrode.

The method of preparing *prothrombin*, by Howell's acetone method, from cell-free citrated dog plasma has been detailed previously (8). The *Ca salt* is N/10 $CaCl_2$. The *thromboplastin* is a saline emulsion (decanted or filtered) of frozen dog brain.

The *fibrinogen* is made from Berkefeld-filtered citrated dog plasma which has stood for several days in the ice-box. A saline solution (pH = 7.5) of the thrice precipitated [$(NH_4)_2SO_4$] protein, when tested with $CaCl_2$ and thromboplastin, shows only traces of clot after many hours, indicating the absence of all but a vestige of prothrombin.

Through the courtesy of Dr. C. H. Best, we have been supplied with purified

heparin from the Connaught Laboratories; 1 mgm. = 110 Toronto units. A 1:1000 stock solution is made in saline, and the pH adjusted to 7.5.

A crude plasma *albumin* is made by precipitating the plasma proteins between 50 and 100 per cent saturation with ammonium sulfate, dialysing away excess of salt, and obtaining a final solution in 0.9 per cent NaCl at pH = 7.5.

Tests. Incubation of thrombic mixtures at less than 10°C. favors stability and lengthens the activation period to the advantage of kinetic study. The clotting-tests proper are made by adding a measured volume of thrombic mixture, 0.5 cc., to fibrinogen, 1.0 cc., plus diluent or inhibitor (2nd-phase control), usually 0.5 cc., at 38°C. Clotting is timed from the first appearance of fibrin strands, assisted, in the prolonged clottings, by gentle agitation of the small (11 mm. diameter) tubes, held in a tilting rack in the thermostatically-controlled water bath. Test conditions are uniform with respect to volumes, temperature, pH, salt and fibrinogen concentration, and each thrombin is tested for stability by repeated C.T. determinations, extending several hours beyond the maximum activation.

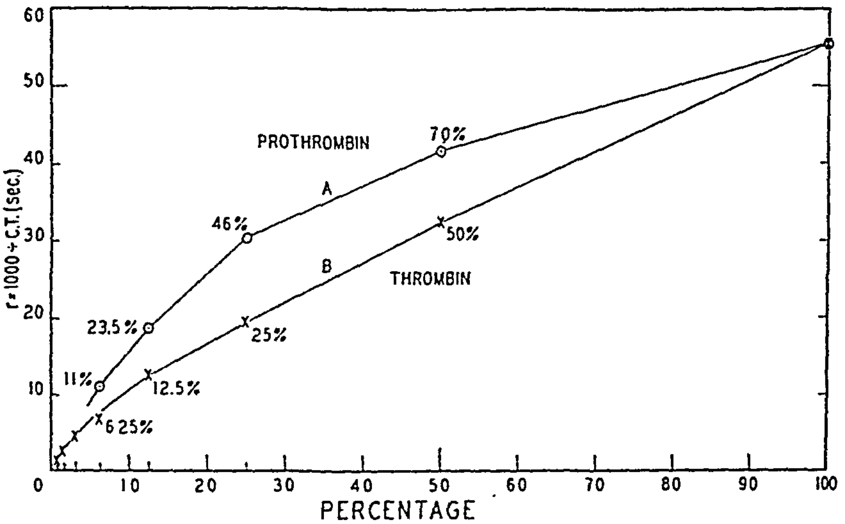

Fig. 1

DATA. Figure 1 illustrates the method of assay of prothrombin in terms of its own reference thrombin. Reciprocal clotting-times r (formulated as 1000 ÷ C. T. (seconds), Fischer, 4) are plotted against dilutions, which are designated as percentages of the original strength. Curve B is the reference thrombin. Curve A is a similar series made after the maximal activation of a corresponding set of prothrombin dilutions. A fixed amount of $CaCl_2$ and brain thromboplastin is added to each diluted prothrombin and the activation to thrombin is followed through the optimum as previously described (8). The respective thrombin "equivalents" are included in the figure. They are obtained by referring the "r" values of A to the reference curve B.

Equivalence of thrombin and prothrombin? There is a marked divergence of the data representing the two series. Obviously, dilution with saline has a different effect upon thrombin and its corresponding prothrombin.

The prothrombin series give more than the theoretical yield of effective thrombin. This has been confirmed on 16 occasions, the only differences being *a*, in two cases, more than a twofold dilution was required to bring out the discrepancy; *b*, in one experiment, only, was there coincidence of the last two or three sets of percentages, suggesting that the possibility of diluting out the divergence is rarely realized.

TABLE 1

Effects of heparin and varying thromboplastin concentration (supra-optimal) on activation of prothrombin to thrombin

pH = 7.5. Activation at 10°C. Clotting-times (sec.) at 38°C.: 0.5 cc. T + 1.0 cc. F + 0.5 cc. saline *or* heparin (C, 2)

	THROMBOPLASTIN DILUTION	INCUBATION PERIOD OF THROMBIC MIXTURE (MIN.)					
		15′	30′	60′	120′	180′	300′
		A. Activation curves, without heparin					
1	1:1	16″	16″	17″	20″		
2	1:2	16″	16″	17″	20″		
3	1:4	40″	16″	16″	20″		
4	1:8	52″	16″	18″	22″		
5	1:16	63″	20″	18″	20″		
6	1:32	160″	50″	18″	19″		
		B. Activation curves, with heparin					
7	1:1	80″	36″	43″	44″		
8	1:2	5°	50″	42″	44″		
9	1:4	∞	36′	43″	43″		
10	1:8	∞	5°	42″	44″		
11	1:16	∞	∞	4°	47″	42″	
12	1:32	∞	∞	∞	3°	75″	40″
		C. Thrombin dilution curves					
		DILUTION					
		1:1	1:2	1:4	1:8	1:16	
1	Saline	17″	30″	44″	75″	126″	
2	Heparin	18″	32″	65″	170″	800″	

As compared with activated plasmas (defibrinated), in which the thrombin formed is very unstable (except in high dilution, 16) because of progressive (serum-) antithrombin (10), our thrombins are sufficiently stable over several hours to discount any possible rôle of thrombinolytic factors. That this is true for the weaker, as well as for the full-strength, prothrombins is borne out by the finding in the data of the subsequent experiments (table 1), which demonstrate that there is no appreciable loss of

potency when prothrombin is activated very slowly, so that any lytic factor present should have plenty of opportunity to affect the first portions of thrombin formed.

Immediate antithrombins (12) can be ruled out, since the same reagents and identical dilutions are used in both thrombin and prothrombin series. Any change in the actual clotting conditions, therefore, must be the result and not the cause of the greater thrombin yields.

By a process of exclusion of known variables, we are led to explain the phenomenon of increase in thrombin yield on prothrombin dilution as due to diminution in the effect of a hitherto unrecognized naturally-occurring *antiprothrombin*. Such an agent has been identified in similar tests on diluted (50–100×) defibrinated (56°C.) plasma. Qualitatively, at least, its action may be compared with the first phase effects of heparin (5).

Mechanism of thrombin formation and the antiprothrombic actions of heparin. Section A of table 1 shows a series of controls in which a single full-strength prothrombin is activated in the presence of various dilutions of brain thromboplastin. The close similarity of the *optimal* clotting-times (16″–18″) is good evidence of the completeness of thrombin formation in all cases. We are dealing, therefore, with a set of conditions under which the prothrombin and calcium (*cf.* 6) are fixed and the thromboplastin always in excess. The only difference between the activation data is in the *rate* of thrombin formation, which is indicated by the length of time required for maximal activation. The weaker the thromboplastin, the longer the period in question.

Section B is a repetition of the foregoing but with an added antiprothrombic factor, namely, a small fixed amount of purified heparin in each thrombic mixture. Dismissing the few seconds discrepancies due to slight thrombin instability (progressive antithrombin, 10), the final clotting-times (circa 40″) are sufficiently uniform to indicate that the conversion, in all cases, is as complete as the new conditions will allow. The most marked action of heparin in the first phase of clotting is the retardation of the prothrombin conversion *rate*, and the clear inverse relation to thromboplastin concentration supports the conclusion that this action is *anti-thromboplastic*.

Sometimes the thrombic potency finally attained in the presence of heparin is identical with the controls (5) but often, as in the present experiments, there is a distinct lessening of optimal potency. This is clearly unrelated to the amount of thromboplastin or rate of thrombin formation. Compare the 40″ (approx.) optima in B with the 16″–18″ in A. The second phase control (C, 1) indicates a negligible effect (1″) on the thrombin-fibrinogen interaction, and there is obviously little or no second-phase cofactor ("pro-antithrombin," 14) or there would be an immediate antithrombic action in the second phase (12). It must be concluded that the 24-

second difference between A and B represents a second component in the first phase action of heparin, namely, a true *anti-prothrombic* effect, manifested by a reduction in the *amount* (effectiveness) of the thrombin formed. In the example cited, this is equivalent, approximately, to a 70 per cent loss of effectiveness.

First phase co-factor for antiprothrombic action of heparin. In contrast to Astrup (1), we always find that the antiprothrombic actions of heparin

TABLE 2

Effects of plasma "albumin" (crude) on the action of heparin in the first and second phases of clotting

Thrombic mixtures (T) = 4 cc. prothrombin + 5 cc. "albumin" (*or* saline) + 0.5 cc. (= 2.5 Toronto *units*) heparin (*or* saline) + 0.25 cc. brain thromboplastin + 0.25 cc. N/10 $CaCl_2$, kept at 7.5°C. for times (min.) stated. Clotting-times (sec.) for 0.5 cc. T + 1.0 cc. fibrinogen + 0.5 cc. saline (*1–5*) *or* 0.5 cc. of a saline mixture containing amounts of heparin, "albumin," *or* both, to make *6*, *7*, *8* equivalent to *2*, *3*, *4*, respectively. C. T.'s at 38°C. and pH = 7.5.

T	INHIBITOR	5′	15′	30′	60′	90′	120′	
		A. Activation curves (first phase)						
1		95″	22″	18″	18″	18″	18″	
2	Heparin	∞	*	900″	30″	23″	23″	
3	Albumin	40″	20″	19″	20″	25″	29″	
4	Hep. + alb.	∞	∞	∞	∞	∞	∞	
		B. Dilution curves (second phase controls, using T_1)						
		DILUTION						
		1:1	1:2	1:4	1:8	1:16	1:32	1:64
5		18″	23″	38″	56″	97″	145″	198″
6	Heparin	19″	30″	55″	235″	*	∞	∞
7	Albumin	18″	25″	39″	61″	100″	150″	260″
8	Hep. + alb.	23″	65″	1030″	1620″	*	∞	∞

* Indicates trace of clot overnight; ∞ = no clot.

in our particular systems (5) require no *added* co-factor, but it remains an open question whether an unknown co-factor accompanies the experimental preparations. Earlier reports (cit. 2) of a first phase co-factor are unconvincing, since the experiments described are not calculated to show up early antiprothrombic action or to rule out immediate antithrombic (second phase) effects. These objections are avoided in the data exemplified in table 2.

The plasma "albumin" (see *reagents*) had no antiprothrombic action but rather a slight *thromboplastic* effect (cf. 7), shown in the early stages of

prothrombin activation. The chosen quantity of heparin had some antiprothrombic action of its own. When the heparin and crude albumin were used in conjuction, however, a marked synergism (potentiation) occurred, with complete inhibition of thrombin formation. The second phase controls show the degree of (immediate) antithrombic action, which, for the selected amounts of the inhibitory agents and full-strength thrombin, is almost negligible in all three cases.

It is concluded that a *first phase co-factor* undoubtedly exists in crude plasma "albumin." It assists heparin to a much greater inhibition of thrombin formation than the second phase co-factor ('pro-antithrombin') contributes to the action of heparin on fully-formed thrombin.

Comment. In spite of demonstrated possibilities of interference by progressive antithrombin, immediate antithrombin (12), and natural antiprothrombin (v. supra), the dilution technique of the Iowa workers (2, 16) is offered as a practical method of "prothrombin" determinations. Our critical analysis is based upon a restricted and modified technique which aims not to serve the purposes, or to question the clinical usefulness, of a general method of assay, but to afford a high degree of control of the cited inhibitors in order to interpret the processes involved in any "prothrombin" method. The above data show that the theoretical basis for definition of assay standards for thrombin and prothrombin should include cognizance of these variables. In the absence of equivalence between prothrombin and thrombin dilutions, it is impossible to assay prothrombin *both* in terms of an arbitrarily fixed clotting-time *and* a definite thrombin dilution value. For practical purposes, it is suggested that at least two clotting-time reference points be selected, with a wide enough range to show up any effects due to the inhibitors mentioned. Pending answer to these suggestions, current "prothrombin" methods must be regarded as empirical and accepted with reservations.

SUMMARY

By quantitatively studying fibrinogen clotting-times with thrombin and prothrombin (maximally activated with *Ca* and thromboplastin), it is shown that the prothrombin dilutions give more than the theoretical yield of thrombin. The thromboplastin variable is ruled out, as it is optimal in all cases. Under these circumstances, thromboplastin variations merely affect the rate of thrombin formation and the optimum clotting-time is a measure of the thrombin yield.

The addition of a fixed amount of heparin slows the rate of prothrombin activation and may result in a lessened amount (effectiveness) of the thrombin formed. The first action is anti-thromboplastic, interpreted as an inhibition of the tryptase-like thromboplastic enzyme (9, 11). The second effect is truly anti-prothrombic, due to an action of prothrombin protein

itself (cf. 4). These two phenomena comprise the "antiprothrombic" (first phase inhibiting) actions of heparin and are easily separable, by control experiments, from the second phase inhibitions, (a) *progressive* (here negligible) and (b) *immediate*. An immediate antithrombin (12) is formed by heparin plus a plasma co-factor (proantithrombin). A similar, but independently acting, *co-factor for the first phase* is demonstrated in crude plasma "albumin." It markedly potentiates the antiprothrombic actions of heparin.

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QUANTITATIVE EFFECTS OF IMMEDIATE ANTITHROMBINS[1]

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In a recent publication in this Journal (3), inhibitors of blood coagulation have been differentiated as follows: 1st phase, antiprothrombins (7); 2nd phase, antithrombins, (a) immediate, (b) progressive (4). This paper deals with quantitative aspects of *immediate* antithrombins, relating their concentration to the relative effectiveness of thrombin as determined by the clotting-time for a test fibrinogen, noting their rôle in thrombin assay, and raising the question of their occurrence in natural clotting systems, including plasma. It has previously been shown that polyvalent anions and also heparin (*plus* its co-factor) antagonize thrombin, whereas cations act directly on fibrinogen (5, 6). The term "immediate antithrombin," therefore, is not so much the designation of a specific thrombin-neutralizing agent as the expression of the ability of the agent to modify the fundamental physico-chemical conditions controlling the thrombin-fibrinogen interaction.

METHOD. Using a standard fibrinogen and a series of thrombin dilutions, the clotting-times (C.T.) afford values for a curve of reference, by which similar data, obtained in the presence of various immediate antithrombins, may be read off as *effective thrombin concentrations* and the degree of inhibition thus measured in terms of the amount of antithrombin used.

Technic. The stability of the thrombin (T) is controlled by keeping a portion of the solution at 38°C. and testing samples at intervals for clotting-power when mixed with fibrinogen solution (F). The clotting is timed from the addition of 1 cc. F to a mixture of 0.5 cc. T and 0.5 cc. saline (0.9 per cent NaCl). The C.T. in these controls must not alter more than a very few seconds in the course of the experiments. In the inhibition tests, the antithrombin, suitably diluted, is substituted for the saline. For the reference curve, the clotting-times of the series of thrombin dilutions are plotted against relative thrombin concentrations, expressed as percentages of the original strength.

Reagents. The citrated dog plasma used in these experiments is preserved by preliminary Berkefeld filtration, a trace of thymol, and storage at 5°C. Fibrinogen

[1] Assisted by a grant from the Horace H. Rackham Research Fund.

is prepared by three precipitations with ammonium sulfate (2). Thrombin is made by the Eagle technic (1): the CO_2-precipitate from diluted plasma is redissolved and activated with $CaCl_2$ and a saline emulsion of dog brain (thromboplastin), defibrination being effected by continual stirring with a glass rod. The thrombin is further purified by acetone precipitation (4). Solutions are made from stock by evaporating off the supernatant acetone (air-jet) and redissolving in 0.9 per cent NaCl solution. The agents to be tested are accurately weighed out and dissolved in distilled water to known volume and strength of solution. The cited concentrations (see tables) refer to amount of agent in final mixture after the addition of all the reagents. Solutions are routinely adjusted to pH=7.4 (glass electrode). Clotting tests are carried out at constant temperature (25°C.) in serological tubes (11 mm. diameter) which are tilted once a second in a mechanical rocker. The clotting is timed from the moment of mixing the thrombin and fibrinogen to the first appearance of fibrin strands (4).

Effects of ferrocyanide, etc., on thrombin clotting of fibrinogen. The immediate antithrombic action of various electrolytes and of heparin was studied for several dilutions of thrombin but a constant amount of fibrinogen. Ferrocyanide was selected as a typical anion of high valency (−4) and a series of dilutions was tested with each thrombin. No significant oxidation-reduction reactions occur under the conditions of these experiments.

In the data graphically presented in figure 1, the various concentrations of ferrocyanide were tested in relation to four different strengths of a thrombin preparation. The figure clearly shows a direct relationship between clotting-time and ferrocyanide concentration for each of the thrombins used. The approximation of the experimental results to a straight line is very satisfactory over a limited range of ferrocyanide and thrombin concentrations, although considerable deviations may occur under some experimental conditions. The *linear relationship* has been found repeatedly within a clotting-time range of about 25 to 120 seconds, i.e., with moderately weak thrombins and not too small amounts of ferrocyanide (see later). It is also evident from figure 1 that the *slopes* of the lines (a) are inversely related to the concentrations of thrombin (E).

K_2SO_4 and KCl have also been studied and found to be much less inhibitory. In using a diluted (1:4) thrombin, the inhibition due to 0.0625 M K_2SO_4 was approximately equal to that of 0.125 M KCl and to that of 0.0039 Molar $K_4Fe(CN)_6$.

Heparin. Heparin (plus its second phase co-factor, *proantithrombin,* 8) resembles ferrocyanide in its relations to fully formed thrombin. Figure 2 represents an experiment in which varying quantities of heparin were tested with four different thrombin dilutions. Plasma, diluted with saline, was used as the source of fibrinogen in order to furnish the heparin cofactor. Sufficient citrate was added to prevent activation of the prothrombin in the plasma.

The results indicate that the clotting-time for each thrombin solution is proportional to the amount of heparin present. Again, the slopes of the

inhibition curves are inversely related to the thrombin concentration. Since data with ferrocyanide (fig. 1) are just as readily obtainable with the use of similar diluted plasma, instead of purified fibrinogen, it is evident that the results of the heparin and ferrocyanide experiments are strictly comparable. The quantitatively analogous behavior affords additional

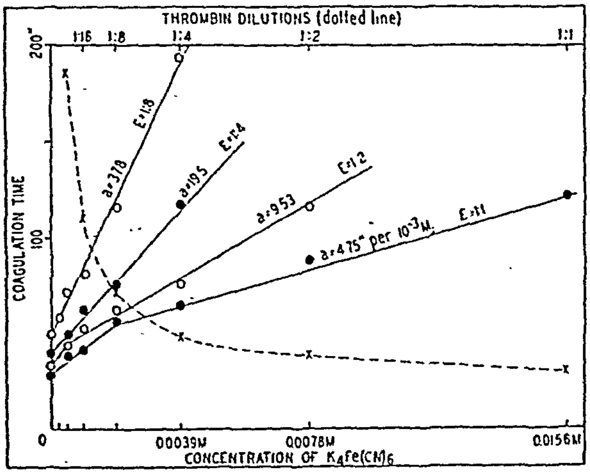

Fig. 1

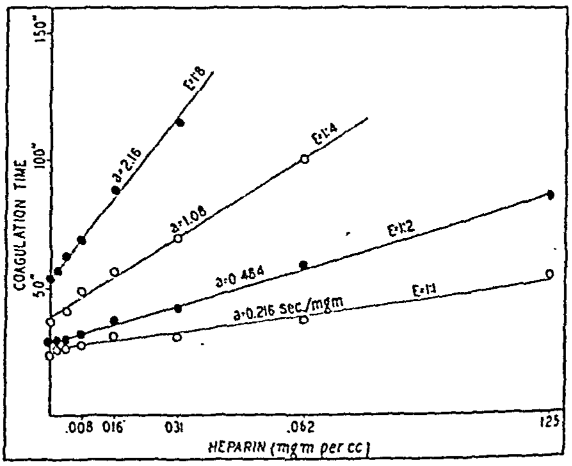

Fig. 2

Fig. 1. Effect of varying ferrocyanide concentration on clotting-times of thrombin-fibrinogen mixtures. Temp. = 25°C.; pH = 7.4. C.T. (sec.) for 1.0 cc. F + 0.5 cc. T + 0.5 cc. inhibitor. Four different thrombin concentrations (E) are tested. The dotted line is a *reference curve*, made with a series of saline dilutions of thrombin, by which clotting-times may be converted into *effective* thrombin concentration, expressed as percentage of original strength.

The linear relation between clotting-time (y) and molal concentration of ferrocyanide (M) may be expressed by the equation

$$y = a\,M + c \ldots\ldots\ldots\ldots 1,$$

where a and c are constants representing, respectively, the *slope* of the "inhibition curve" and the Y-axis intercept. The inverse relation of slope to thrombin concentration (E) may be expressed by the differential equation

$$dy/dM = K/E \ldots\ldots\ldots\ldots 2,$$

where K is a constant. The differential factor (dy/dM) is identical with slope a, for all values of M, when the direct relationship expressed by equation 1 holds.

Fig. 2. Effect of varying heparin concentration on clotting-times of mixtures of citrated plasma and four different thrombin concentrations. Same conditions and analysis as in figure 1.

support for the conclusion that heparin and polyvalent anions affect coagulation through a fundamentally similar mode of action (5).

Assay of thrombin and prothrombin. We have noted that the *slope* of the inhibition curve (with ferrocyanide, for instance) is an index of thrombin concentration. Although difficulties are to be anticipated in practice, this offers a new principle for thrombin, and prothrombin, assay.

Naturally-occurring immediate antithrombins in plasma. Table 1 illus-

trates tests made with a single thrombin preparation and three different coagulable solutions: (a) purified fibrinogen, (b) dog plasma I, from a healthy animal under nembutal anesthesia, (c) dog plasma II, from an animal under morphine-urethane anesthesia and in a condition of shock due to prolonged experimentation with vasomotor drugs. At the same time (expt. D) the thrombin was tested on fibrinogen in the presence of varying concentrations of potassium ferrocyanide. Although the plasmas

TABLE 1

Assay of the immediate antithrombins of plasma

	THROMBIN DILUTION									
	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
A. Fibrinogen.......	11″	13″	16″	20″	25″	35″	52″	75″	115″	∞
B. Plasma I (1:10)..	13″	16″	20″*	27″	42″	60″	87″	95″	120″	200″
C. Plasma II (1:10).	60″	55″	48″†	52″	55″	58″	80″	104″	∞	∞
D. $K_4Fe(CN)_6$.......	M/8	M/16	M/32	M/64	M/128	M/256	M/512	M/1024	M/2048	M/∞
Fibrinogen+ thrombin (1:4)	100″	70″	55″	46″†	39″	34″	31″	28″	25″	22″*

Five-tenths cubic centimeter thrombin is used to clot 1 cc. of plasma or fibrinogen, as indicated, in the presence of 0.5 cc. of the specified concentration of ferrocyanide (D) or of 0.9 per cent saline (A, B, C).

* and † indicate approximately equivalent clotting-times (see text).

TABLE 2

Anomalous effects produced by dilution and inhibitors

A. Thrombin dilution.........	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Clotting-time (C.T.)......	36″	15″	13″	17″	22″	40″	60″	120″

B. $K_4Fe(CN)_6$................	M/∞	M/2560	M/1280	M/640	M/320	M/160
C.T. (thrombin 1:1).......	36″	32″	32″	30″	47″	240″
C.T. (thrombin 1:2).......	15″	18″	20″	24″	28″	115″

Clotting tests: 0.5 cc. saline or ferrocyanide + 1.0 cc. diluted plasma (with added citrate) + 0.5 cc. Eagle-type thrombin.

are similarly diluted and citrated, they show a marked difference in coagulability on adding the various thrombin dilutions. A somewhat crude comparison (since some minor variables, such as fibrinogen concentration and amount of citrate (in fibrinogen), were not controlled) may be made by comparing the clotting-times with 1:4 thrombin, referring each to the ferrocyanide series, as an empirical standard of assay. This gives the finding of about 64 times as much inhibitor in plasma II as in plasma I, which may be taken as indicative of a striking increase in *immediate antithrombin* in the plasma from the animal in shock.

Anomalous clot-acceleration by "inhibitors". It is not an infrequent finding (table 2) that very small amounts of ferrocyanide shorten the clotting-time of thrombin-fibrinogen mixtures, in marked contrast to the inhibition seen with greater concentrations. The same result is obtained with many plasmas, the coagulation of which is timed from the addition of Ca-thromboplastin in the presence of weak ferrocyanide. In experiments C (table 1) and A (table 2) is seen an occasional anomaly, namely, an improvement in clotting-power on diluting a thrombin preparation. The suggested explanation in all these cases is an improvement in the physical conditions for clotting.

Discussion. The linear relationship which is demonstrated between clotting-times and concentration of antithrombin is significant because it indicates that the reaction between thrombin and fibrinogen is diminished by a definite amount, which can be directly related to concentration of inhibitor. From the quantitative relations established between clotting-time, strength of thrombin, and concentration of (immediate) antithrombin, it is theoretically possible to determine any one of these variables if the other two are known. Potassium ferrocyanide is an easily reproducible standard of reference for the assay of the second phase actions of heparin and perhaps for naturally-occurring inhibitors of the same general type.

SUMMARY

A direct relation is found to exist between the clotting-time (thrombin + fibrinogen-containing fluids) and the concentration of inhibitors (electrolytes or heparin plus co-factor).

The rate of increase of clotting-time with increasing concentrations of these antithrombins, i.e., the "inhibition slope", varies inversely with the thrombin concentration.

The principles of new methods for the assay of thrombin and immediate antithrombins are outlined. Potassium ferrocyanide is suggested as a reference standard for the latter.

A clot acceleration effect produced by traces of immediate antithrombins, or, occasionally, by dilution, is observed with plasma and with some thrombin preparations.

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THE RELATIVE RESPONSES OF THE DORSAL METACARPAL, DIGITAL AND TERMINAL SKIN ARTERIES OF THE HAND IN VASOCONSTRICTOR REFLEXES[1]

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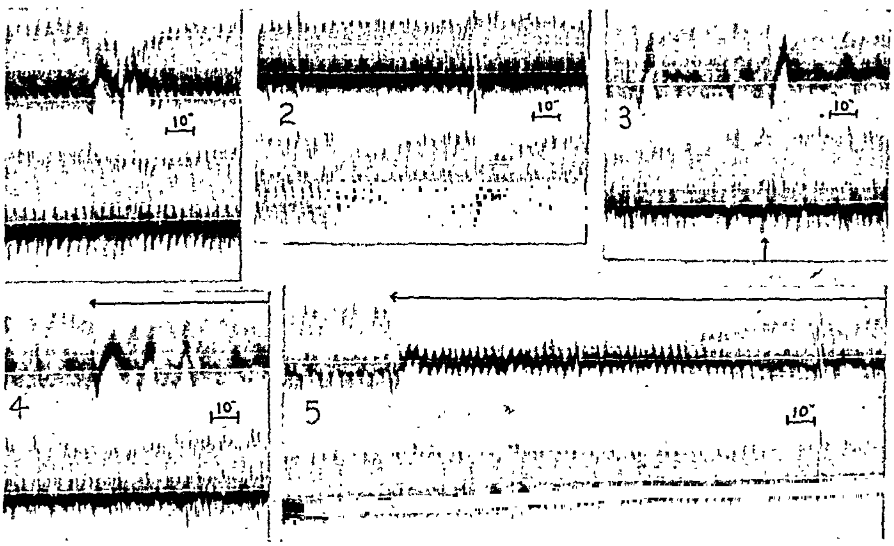
The interpretation of the effects of cold on the finger circulation indicated the advisability of extending observations on arterial reactions in the hand to include the intermediate arteries lying between the radial and ulnar arteries and the minute terminal arteries of the finger pad. The possible participation of the digital, metacarpal and volar arch arteries in the vascular reactions in the hand is not indicated in the usual experiments done on the finger or hand circulations. It is usually assumed without an adequate observational basis that the vasomotor discharges to the hand involve chiefly the terminal arterial branches since these have the most abundant innervation and, to a less extent, the larger hand arteries since these receive fewer fibers. The differences in reaction would according to this assumption be quantitative rather than qualitative. However, observations on the radial artery and the finger pad (1) where the argument applies similarly indicated that profound constriction could occur in the finger pad without effect on the radial artery. This implies a selective discharge in the vasomotor system.

Can this discharge to the hand arteries be so selective that it can involve only the terminal skin arteries without effect on the tone of the intermediate hand arteries? This paper answers the question in the affirmative.

METHOD. The reactivity of the larger arteries of the hand has been studied by recording their volume pulses with the photoelectric plethysmograph (2). The volume pulses of the dorsal metacarpal arteries and of the A. volaris indicis radialis may often be recorded with ease without much interference from the terminal skin arteries which are far less abundant on the dorsal aspects of the hand (3). Thus, when the plethysmograph is moved only slightly from the position where the artery's pulse is readily

[1] This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association. Generous assistance from the Burgess Battery Company, Freeport, Illinois, has also been received and is gratefully acknowledged.

recorded, the pulsations decrease sharply in magnitude and become difficult to record at all. This conveniently eliminates any significant error due to the pulsations at the small skin arteries. Convenient positions for the plethysmograph are at the base of the index finger on its radial aspect and in the fork between the index and middle fingers. In neither position are the plethysmograms much affected by reactions in the palmar skin. Occasionally, the first dorsal metacarpal artery (branch of the radial) lies so close to the skin surface that its pulsations are readily palpated and recorded. The digital artery pulses are usually readily recorded by placing the plethysmograph near or over the first interphalangeal joint some-



Figs. 1–5. Volume pulses of finger pad and of dorsal metacarpal artery. Time: 10 seconds.

Fig. 1. Spontaneous waves. Upper record of pad.

Fig. 2. Spontaneous waves. Raynaud's disease. Lower record of pad.

Fig. 3. First constriction is spontaneous. Second constriction is in response to a loud noise. Upper record of pad.

Fig. 4. Immersion of opposite hand in ice water. Upper record of pad.

Fig. 5. Application of cold to finger whose pad pulse is recorded in upper record.

what toward the palmar surface. Occasionally the digital artery records are mixed with effects from the terminal arteries in the pad. When this is the case, mild vasoconstrictor stimuli *seem* to elicit constrictions in the digital artery as well as in the pad. A slight shift in the position of the plethysmograph will eliminate these responses and also result in a large increase in the amplitude of the recorded pulse.

Subjects (healthy adults unless otherwise noted) were in a sitting position with the arm flexed and raised slightly. This position seems to provide the best protection against gross artifacts due to movements.

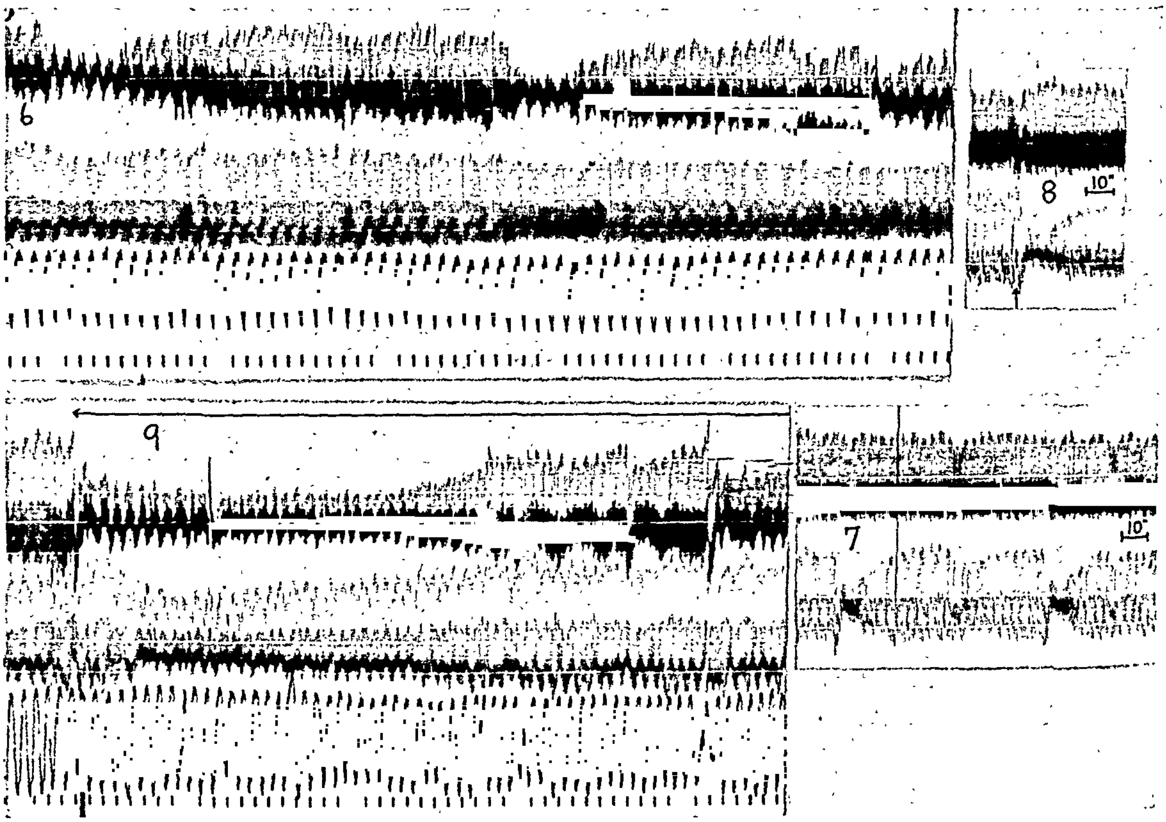
RESULTS. The dorsal metacarpal arteries are smaller and better muscled

tubes than the radial and ulnar arteries and their immediate branches. One might therefore expect the dorsal metacarpal arteries to participate more frequently in the vasoconstrictor reflexes involving the small arteries and arterioles of the finger skin but such has not been our experience (figs. 1–5). This is particularly true when the vasoconstrictor discharges are brief as in the spontaneous waves of short duration (figs. 1 and 2), where complete absence of constrictor effect on the metacarpal arteries is usually the case. It was interesting to note that this was also true in similar observations on a case of Raynaud's disease (fig. 2). Occasionally more prolonged discharges may slightly affect the metacarpal artery pulse. The vasoconstrictor reflexes elicited by loud noises (fig. 3), by deep breaths, by immersion of the opposite hand in ice water (fig. 4) were usually without effect on the amplitude of the metacarpal artery pulses. A well sustained constriction may be elicited in the finger by local cold to it without any indication of a constrictor effect on the supplying metacarpal artery (fig. 5). The cold stimulus regularly elicits constriction in the unchilled finger as well, so the failure of the metacarpal artery to constrict must in this case be due to the selective character of the vasoconstrictor discharge. The usual absence of vasoconstrictor reflexes in the metacarpal arteries parallels the observations on the radial artery (1). In a few instances where a moderate participation of the metacarpal arteries in the vasoconstrictor discharges seemed to occur, the plethysmograph was placed at the fork of the second and third fingers and directed towards the palmar skin. One could not then be quite certain that the volume pulse record had not been directly affected by reactions of the small arteries and arterioles in the trans-illuminated skin.

The results on the digital arteries were somewhat surprising when considered from the anatomical viewpoint. These vessels have a fairly thick media, they receive a vasomotor innervation and they can go into powerful spasms (4). In view of the extreme lability of the finger circulation, the ease with which vasomotor reflexes can be demonstrated here, and the extraordinarily large changes which can and do occur in the finger's blood flow (5), one may expect vasoconstrictor reflexes to involve the digital arteries as well as the minute vessels of the pad. Only quantitative but not qualitative differences in the responses of these finger vessels may be expected if Schretzenmayr's thesis of synergistic participation of the larger arteries in the vasomotor reflexes (6) is ordinarily applicable to man. No better test of this idea could be made than in the extremely labile vascular field of the finger.

The results (figs. 6–9) indicate that the digital arteries are not usually involved in the vasoconstrictor discharges to the finger. Thus, spontaneous waves (figs. 6 and 7) and the reactions in the pad vessels to such stimuli as loud noises (fig. 8) may be and usually are without any constric-

tor effect on the digital artery. In instances of apparent participation in such reflexes one is justified in questioning the validity of the observations since exact placement of the plethysmograph is required if the pad pulses are not to affect the digital artery records. The small vessels of the pads of the three phalanges participate regularly in the vasomotor reactions of



Figs. 6–9. Volume pulses of finger pad and of digital artery at first interphalangeal joint.

Fig. 6. Spontaneous waves. Upper record of pad. Respiration and time in 5 seconds.

Fig. 7. Spontaneous waves. Lower record of pad.

Fig. 8. Loud noise. Lower record of pad.

Fig. 9. Application of cold to finger whose pad pulses and digital artery pulses are being recorded. Upper record of pad. Respiration and time in 5 seconds.

the terminal phalanx. We have seen no exceptions to this statement in healthy subjects.

The application of cold to the finger in which the reactions of the pad vessels and of the digital artery are being recorded should be an excellent test of the participation of the latter in vasoconstrictor reflexes in the finger. When this is done by methods which will be described elsewhere, the digital artery may fail to constrict until its temperature has fallen

(fig. 9). The initial constriction in the pad of the chilled finger is due to vasoconstrictor reflexes elicited by the cold and acting on the warm fingers of both hands as well as on the chilled finger. (The detailed description of these responses will appear in a following paper.) The digital artery volume pulses may increase at the moment constriction occurs in the pad. This may be the result of a rise in pressure in the digital artery due to constriction in the arterioles which it supplies. As chilling proceeds, the digital artery volume pulses may decrease somewhat (fig. 9), due possibly to a direct constrictor effect of cold on the artery.

COMMENT. These experiments are in agreement with the generally held opinion that the vasomotor reflexes act principally on the arterial gateway, the small arteries and arterioles. They also follow logically from the previously published observations on the reactions of the radial artery in similar circumstances (1) and they add evidence in support of the position taken there.

There is nothing in the known anatomy of the sympathetic nervous system and of the hand arteries which would lead one to expect that a vasomotor reflex to the arterioles and small arteries of the skin would be limited to these small vessels and would not also involve the larger arteries of the hand. Failure of the latter vessels to be included in the vasoconstrictor reflexes in the hand circulation can not be due to lack of muscle in the walls of these arteries or to the absence of an innervation. Quantitative differences between the responses of the digital arteries and those of the smaller arteries and arterioles which they supply may be expected but complete absence of a vasoconstrictor response in the digital artery at the time that its minute branches constrict powerfully is another matter. It should be emphasized that this may happen at the time that the vasoconstrictor discharges are sufficiently widespread to involve fingers and toes.

The simplest position is to consider these results as further evidence that the vasomotor reflexes are highly selective with respect to the vascular topography involved in the reactions, that these ordinary vasomotor activities are not the result of mass actions by the sympathetic nervous system. We so interpret the data of this paper.

There is nothing implied in this interpretation which would in any way negate the possibility of the larger hand arteries and the larger main arterial trunks of the arm of participating in more massive disturbances of the arterial tree than those described here.

SUMMARY

The participation of the intermediate hand arteries, the dorsal metacarpal arteries and the digital arteries, in the vasoconstrictor reflexes of the hand, has been studied by recording their volume pulses with the photoelectric plethysmograph.

These arteries do not usually participate in the so-called spontaneous waves (figs. 1, 2, 6 and 7), in the vasoconstrictor reflexes elicited by loud noises (figs. 3 and 8), by immersion of opposite hand in ice water (fig. 4), or by application of cold to the finger whose pad pulses are being recorded (figs. 5 and 9).

These results are most simply explained by considering the vasomotor reflexes as highly selective with respect to the vascular topography involved in the reactions.

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THE CIRCULATORY RESPONSES OF CHRONIC SPINAL DOGS TO ETHER ANESTHESIA[1]

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The fall in mean arterial blood pressure which occurs when ether is administered to sympathectomized dogs (1) suggests that during ether anesthesia the activity of the sympathetic nervous system is essential for the maintenance of the normal blood pressure level. Since it is known that the isolated spinal cord can mediate some sympathetic activity in response to various types of stimuli (2, 3, 4), it seemed of considerable interest to compare the circulatory responses to ether inhalation shown by chronic spinal dogs with those exhibited by sympathectomized dogs.

METHODS. The data reported in this study have been gathered from experiments on seventeen spinal dogs. All operations were performed aseptically under nembutal anesthesia. The animals were used for the ether experiments four to fifty-three days after transection of the spinal cord, the duration of the postoperative period not significantly altering the results. At the time of experimentation, the animals were in good condition. In every instance the level of the cord section was ascertained by autopsy. The sites of the spinal cord sections are shown in table 1.

The mean arterial blood pressure was determined in the femoral artery by the insertion of a glass cannula connected to a mercury manometer. The heart rates were counted from the kymograph records and checked against rates counted with a stethoscope. Blood ether concentration was measured by Ruigh's modification of the iodine pentoxide method (5). Two cubic centimeters of jugular vein blood were used for each determination.

After a control period of from one-half to one and one-half hours of rest on the animal board, the dogs were etherized by the drop method for one hour.

RESULTS. As in the experiments with sympathectomized dogs (1), only the stage of excitement and the stage of surgical anesthesia were recognized. The events occur so quickly in the dog that it is impractical to distinguish the first and fourth stages.

[1] A preliminary report of this work was presented before the American Physiological Society, Toronto, This Journal **126**: P613, 1939.

Dogs with spinal cord section below T-10. In animals with spinal cord section below T-10 (table 1), the effects of etherization on blood pressure and heart rate were the same as in normal animals (1). Figure 1 shows a blood pressure tracing from one of these animals.

Dogs with spinal cord section above T-7. In thirteen experiments on nine dogs with cord section above T-7 (table 1), the control values for ar-

TABLE 1

The distribution of the spinal cord sections

NUMBER OF ANIMALS	LEVEL OF CORD SECTION
1	7th to 8th C
2	8th C to 1st T
8	1st to 2nd T
2	4th to 5th T
1	6th to 7th T
1	11th to 12th T
1	13th T to 1st L
1	1st to 2nd L

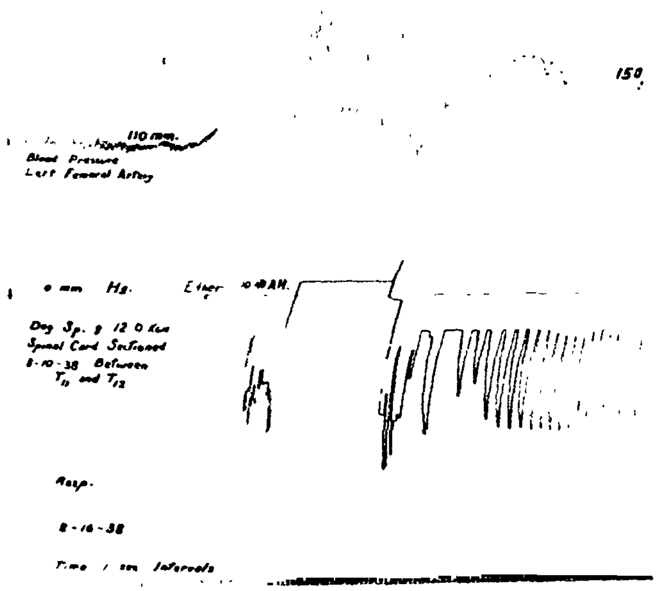

Fig. 1. The effect of ether inhalation upon the blood pressure and heart rate of a dog six days after the spinal cord had been sectioned between T-11 and T-12.

terial pressure were slightly below normal (average 95 mm. Hg), but the resting heart rates fell within the normal range (80 to 100). In striking contrast with the effects on normal and low spinal dogs, the induction of anesthesia in high spinal animals (fig. 2) caused in every instance a precipitous fall of 30 to 60 mm. Hg in arterial blood pressure accompanied by bradycardia. As the surgical stage was entered, the blood pressure showed

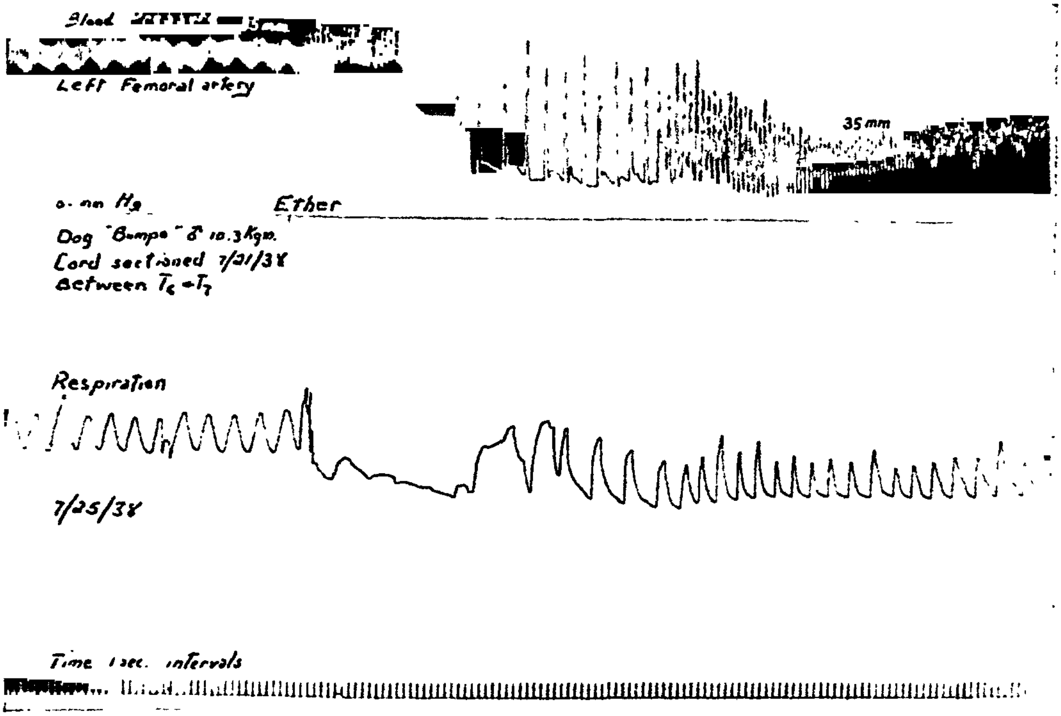

Fig. 2. The effect of ether inhalation upon the blood pressure and heart rate of a dog four days after transection of the spinal cord between T-6 and T-7.

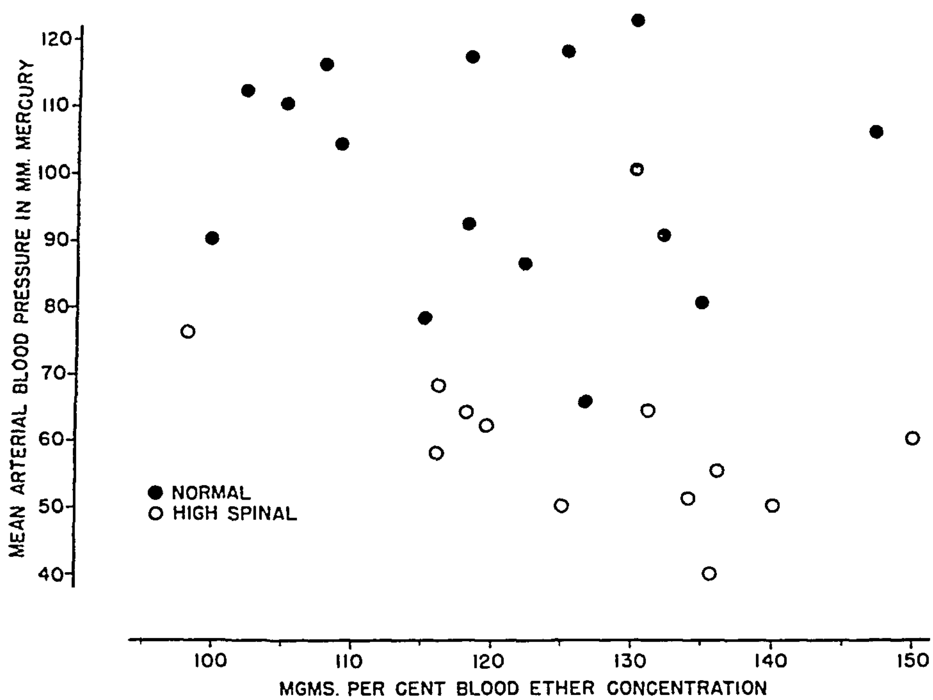

Fig. 3. The relation of the mean arterial blood pressure to the blood ether concentration in normal and high spinal dogs.

only a slight tendency to return to the control value. During full surgical anesthesia, the mean blood pressure levels usually ranged between 40 and 65 mm. Hg (fig. 3). Under these conditions the acceleration in heart rate varied with the level of cord transection. Thus, with sections at or above T-1, the rate increased to only about 135 beats per minute (the denervated heart rate), whereas with sections below T-4 the rate increased to approximately 200, as in normal animals.

The vagi were cut in three dogs with spinal cord sections between C-6 and T-1. In these animals, the administration of ether caused an abrupt fall in blood pressure without bradycardia (fig. 4). This response closely resembles that shown by sympathectomized, vagotomized dogs (1).

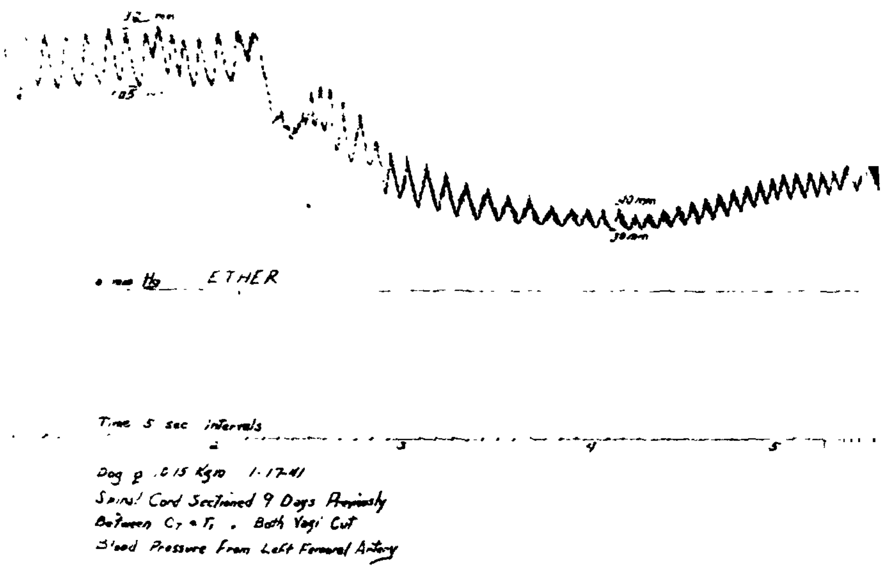

Fig. 4. The effect of ether inhalation upon the blood pressure of a bilaterally vagotomized dog nine days after the spinal cord had been cut between C-7 and T-1.

During surgical anesthesia, the blood pressures and the heart rates of vagotomized, high spinal dogs were the same as those shown by high spinal dogs with intact vagi.

The effect of ether anesthesia upon the circulation has also been studied in spinal cats. Etherization of four cats with spinal cord sections above the eighth thoracic segment caused the same blood pressure and heart rate responses that were observed in the high spinal dogs. In two cats with cord section below the ninth thoracic segment, the administration of ether produced circulatory changes identical with those shown by normal cats and dogs.

Discussion. During the excitement stage of ether anesthesia, spinal dogs exhibit the bradycardia shown by normal and sympathectomized

dogs. This response, which is absent in vagotomized, spinal dogs, is attributed to the reflex stimulation of the vagal centers by the irritant action of ether upon the respiratory mucosa (6).

During the surgical stage of ether anesthesia (blood ether, 100 to 150 mgm. per cent), the heart rate depends upon the level of the spinal cord transection. As in normal dogs, the heart rate of animals which have suffered section of the cord below the fourth thoracic segment increases to about 200 beats per minute. But if the upper thoracic segments are cut off from suprasegmental control, less cardiac acceleration is produced by surgical ether anesthesia. In dogs with low cervical transections, the heart rate increases during surgical anesthesia to about 135 beats per minute. This is the rate of the denervated heart and indicates that the rate has increased only to the extent that can be accounted for by the removal of vagal inhibition. When the vagal effects are eliminated by bilateral vagotomy, the induction of ether anesthesia causes no change in heart rate (fig. 4). These findings indicate that the isolated sympathetic cardio-accelerator centers of the spinal cord are not excited by ether.

The rise in blood pressure shown by normal dogs during the excitement stage of ether anesthesia (1) does not occur when the suprasegmental control of the spinal sympathetic centers below the sixth thoracic segment is cut off by spinal cord section at or above that level (see figs. 1 and 2). Instead, these high spinal animals show a fall in pressure similar to that exhibited by sympathectomized dogs (1). On the other hand, the administration of ether to animals with cord transections below the tenth thoracic segment produces the rise in blood pressure observed in normal dogs (fig. 1). The same relations prevail under surgical ether anesthesia. With cord sections above the seventh thoracic segment the blood pressure is low (30–60 mm.), whereas, with cord sections below the tenth thoracic segment, the blood pressure remains within the normal range (100–120 mm.) (see figs. 1 and 2). Thus, it is apparent that an important sympathetic outflow for the nervous control of blood pressure during ether anesthesia lies between the sixth and the eleventh thoracic segments.

The different vascular responses shown by high and low spinal dogs suggest that the splanchnic nerves may be of major importance in the maintenance of the normal blood pressure. In view of this, bilateral subdiaphragmatic splanchnic nerve section was performed on two dogs. The three experiments on these animals showed that ether anesthesia produced blood pressure and heart rate changes identical with those of normal animals except that the rise in pressure during the excitement stage was less pronounced. Hence, it seems that in the absence of the splanchnic nerves impulses mediated by other sympathetic vasoconstrictor fibers are able to maintain the vascular tone.

Since this study does not include measurements of the cardiac output,

an accurate evaluation of the blood pressure changes is not possible. Nevertheless, the direct relation between blood pressure variations and the amount of the sympathetic nervous system which remains connected with the suprasegmental centers suggests that the observed effects result from alterations in peripheral resistance rather than from primary changes in cardiac output. Assuming that the mechanism of peripheral vascular reactions is identical in etherized, high spinal dogs and in etherized, sympathectomized dogs, vascular dilatation may be produced either by stimulation of dilator mechanisms in the cord or by the direct action of ether or certain metabolites upon the blood vessels. In the normal animal, such dilatation is doubtless masked by sympathetic vasoconstrictor impulses originating above the cord.

SUMMARY

1. The administration of ether to dogs with the spinal cord sectioned below the tenth thoracic segment produces the same changes in blood pressure and heart rate that are shown by normal animals (fig. 1).

2. The inhalation of ether by dogs with the spinal cord cut above the seventh thoracic segment results in an immediate fall in blood pressure. This is associated with a marked bradycardia (fig. 2). During surgical anesthesia the mean blood pressure ranges between 40 and 65 mm. Hg (fig. 3). In this stage the degree of cardiac acceleration is related to the amount of the residual cardio-accelerator outflow which remains connected with suprasegmental centers.

3. The administration of ether to vagotomized, high spinal dogs (cord section C-6 to T-1) causes no change in heart rate and produces the same blood pressure responses that are shown by high spinal dogs with intact vagi (fig. 4).

4. Etherization of dogs with bilateral splanchnic nerve section produces in general the circulatory responses observed in normal dogs.

5. It is concluded that for the maintenance of normal blood pressure during ether anesthesia, the suprasegmental control of the sympathetic nervous system must extend below the sixth thoracic segment.

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THE EFFECT OF DOSAGE AND DURATION OF ADMINISTRATION ON THE ANTI-UREMIC EFFECT OF DESOXYCORTICOSTERONE

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Selye (1) and Ludden et al. (2) reported that desoxycorticosterone acetate (D.C.A.) has a renotropic action which is very similar to that of testosterone and progesterone. This kidney-stimulating effect has been proven to be direct and not mediated by the hypophysis since all these steroids are active in the absence of the pituitary (3). However, since they are not as active as in the intact animal and fail to restore the atrophied kidneys of the hypophysectomized rat to their normal size, Selye concluded that most probably the kidney is dependent for the maintenance of its normal structure on the balanced production of other hypophyseal principles (growth and thyrotropic?) in addition to the luteinizing and adrenotropic hormones. Selye (4) has observed a beneficial action of both testosterone and D.C.A. on the kidney after sublimate poisoning; but even more remarkable was the constant protective effect obtained with D.C.A. in experimental uremia produced by complete nephrectomy since this action could not merely be due to the renotropic effect of the steroid (5). Furthermore, Selye and Nielsen (6) reported that D.C.A. not only prolongs the survival time and delays the clinical signs of uremia, but actually inhibits the rise in the non-protein nitrogen content of the blood after complete nephrectomy. Since several investigators have already discussed the possibility that because of their renotropic effect certain steroids may be useful in the clinical treatment of kidney disorders, this action of D.C.A. appears to be particularly important. The use of this substance in place of androgens has the definite advantage of eliminating specific effects on the sex organs. Therefore it was deemed worthwhile to investigate what would be the optimum length of pretreatment and the optimum dose for the experimental study of the antiuremic properties of D.C.A. in mice and rats.

In order to establish these conditions we performed a number of experiments on female albino mice with body weights averaging 18 grams. In the first experiment we attempted to establish the optimum daily dose, and, as shown in the table, groups 1 to 6 received subcutaneous injections

of D.C.A. dissolved in 0.2 cc. of peanut oil in doses ranging from 0.1 to 5.0 mgm. The right kidney was removed after careful decapsulation so as to prevent any injury to the adrenal and 24 hours later pretreatment was started. Six days having been tentatively chosen for the length of pretreatment, the left kidney was removed at the end of that period and the survival time of each animal was recorded. The results summarized in table 1 indicate that all doses above 0.1 mgm. significantly increase the

TABLE 1

Survival time of nephrectomized mice and rats after pretreatment with D.C.A.

	GROUP	DAILY TREATMENT	NUMBER OF DAYS	NUMBER OF ANIMALS	MEAN BODY WEIGHT	SURVIVAL TIME		P*
						Mean	Range	
					grams	*hours*		
I	1	0.2 cc. oil	6	9 mice	17	26	20-36	
	2	0.1 mgm. DCA	6	10 mice	18	32	24-38	0.06
	3	0.5 mgm. DCA	6	10 mice	17	34	23-50	0.05
	4	1.0 mgm. DCA	6	10 mice	17	35	22-50	0.05
	5	2.0 mgm. DCA	6	10 mice	17	35	22-46	0.03
	6	5.0 mgm. DCA	6	10 mice	18	34	23-48	0.05
II	7	0.1 cc. oil	6	10 mice	17	25	19-41	
	8	2.0 mgm. DCA	1	12 mice	18	28	19-45	0.40
	9	2.0 mgm. DCA	3	11 mice	17	43	35-46	<0.01
	10	2.0 mgm. DCA	6	11 mice	19	34	22-47	0.03
	11	2.0 mgm. DCA	12	10 mice	19	36	22-48	0.02
	12	2.0 mgm. DCA	24	10 mice	19	38	23-47	0.02
III	13	0.1 cc. oil	3	12 mice	17	30	24-40	
	14	2.0 mgm. DCA	3	12 mice	18	48	40-56	<0.01
	15	2.0 mgm. DCA	6	12 mice	18	38	26-51	0.01
IV	16	0.2 cc. oil	3	12 rats	90	33	29-39	
	17	2.0 mgm. DCA	3	12 rats	96	36	31-42	0.05
	18	5.0 mgm. DCA	3	12 rats	95	41	33-52	<0.01

* Probability calculated from Fisher's table of t (7). Results are considered to vary significantly from the normal when P is equal to or smaller than 0.05.

survival time, and the optimum daily dose of D.C.A. in mice is 2.0 mgm. since group 5 has the lowest P value combined with the longest mean survival time.

Having obtained an approximate optimum daily dose of D.C.A., we performed a second experiment (groups 7 to 12) in which all animals were treated with the dose of 2.0 mgm. and the length of pretreatment varied from 1 to 24 days. In all other respects the experiment was conducted as in the previous series, the right kidney being removed 24 hours before the

first injection and the left kidney on the last day of pretreatment. Here we found a significant difference in the length of survival of the different groups wherever the number of days of pretreatment was at least 3, with an optimum value for 3 days where P was less than 0.01 and the mean survival time longest.

A third similar experiment (groups 13 to 15) emphasized these results giving as the optimum daily dose for mice 2.0 mgm. of D.C.A. and as the optimum length of pretreatment 3 days.

After having ascertained optimum conditions in the mouse, we attempted to do the same in the rat. For this purpose we performed a fourth experiment (groups 16 to 18) on 36 albino rats with 6 males and 6 females in each group, all having an average weight of 95 grams. All groups were pretreated for 3 days, one with 0.2 cc. of peanut oil, one with 2.0 mgm. and the others with 5.0 mgm. of D.C.A. daily subcutaneously. The best among the doses tested was the 5 mgm. daily dose with 3 days' pretreatment.

SUMMARY

Desoxycorticosterone acetate exerts a protective effect in experimental uremia produced by complete nephrectomy and although it does so when given in ranging doses and for different periods of time, the optimum conditions for the study of these antiuremic properties have been found to be 2.0 mgm./day in the 18 gram mouse, and 5 mgm./day in the 95 gram rat given subcutaneously in oil for 3 days.

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VARIATIONS IN FILLING AND OUTPUT OF THE VENTRICLES WITH THE PHASES OF RESPIRATION

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Several workers have made use of the cardiometer for studying respiratory effects on ventricular filling and output. The application of their findings to normal breathing is, however, somewhat questionable. Henderson and Barringer (1913b) used dogs with bilateral open pneumothorax. The lungs were kept inflated by a stream of air under positive pressure, controlled by a Müller water valve. The rate of insufflation was so adjusted that the animal continued to make rhythmic respiratory movements, but the intrathoracic pressure was at least atmospheric and the intrapulmonary pressure somewhat higher. Eyster and Hicks (1933) and Cahoon, Johnson and Michael (1940), after applying the cardiometer, closed the chest so that natural breathing could go on; but the cardiometer was connected to a recording device moving in outside air. The ventricles thus had to fill against an unvarying atmospheric pressure. Eyster and Hicks recognized this as a possible source of error, but did not consider it to be of critical importance. The ventricles, because of their thick walls, were believed to be little if at all affected by the normal respiratory changes of external pressure upon them. This assumption is not uncommon in the earlier literature, but it seems always to have lacked experimental support, and is shown below to be untenable. In the present study we have used a recording system in which external pressure on the ventricles is always approximately equal to intrathoracic pressure, varying as the latter does with the phases of respiration.

APPARATUS. The arrangement is shown diagrammatically in figure 1A. The recording device is a tambour with a wide membrane, inclosed on both sides. The air chamber on one side is connected to the cardiometer, that on the other to the intrapleural space of the thorax. Except for these connections the chest is closed, and the animal breathes naturally. The side outlets at C_1 and C_3 are shut off by clamps after initial adjustment of the air content on both sides of the membrane. On the cardiometer side, the volume should be so regulated that the excursions to be recorded are approximately equal in both directions from the plane position of the

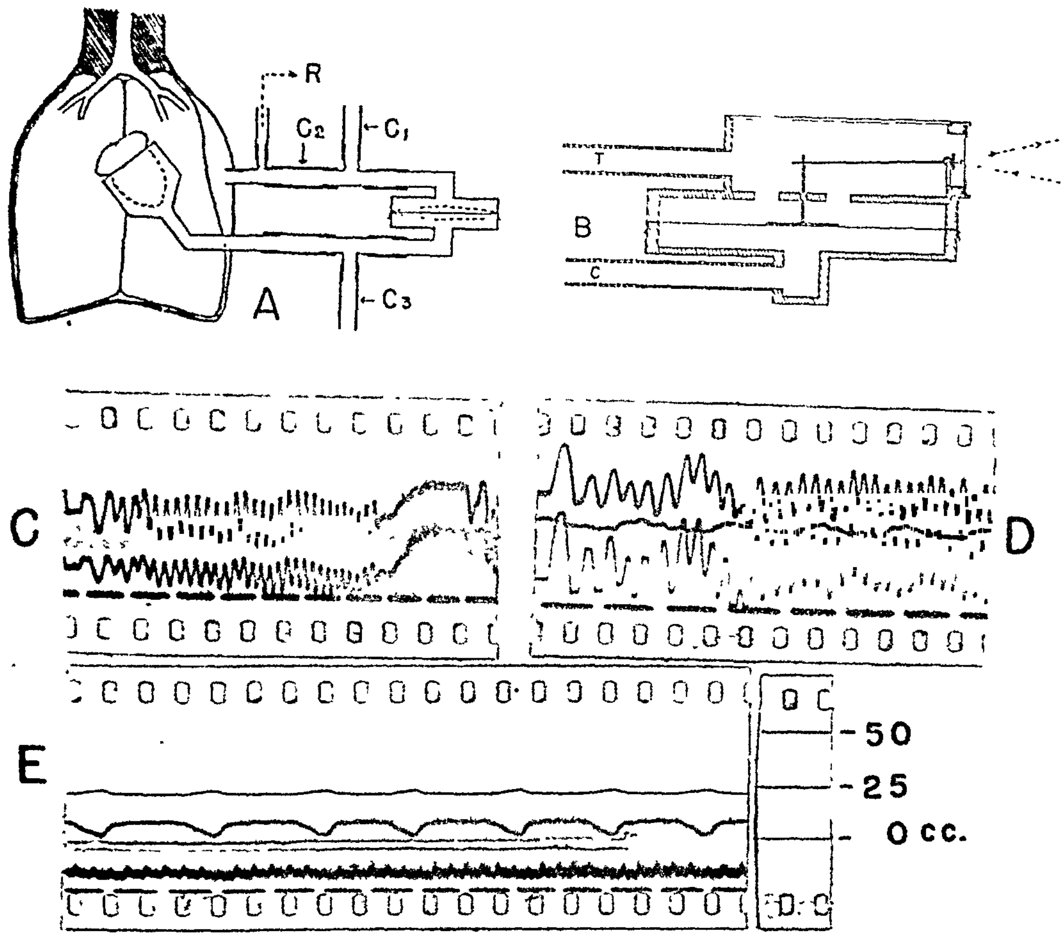

Fig. 1. A. Diagram of recording system. *R*, outlet to segment capsule recording respiration. C_1, C_3, side outlets which are clamped off after initial adjustment of air volume on both sides of the tambour membrane; C_2, alternative position for clamp at C_1. Further explanation in text.

B. Recording tambour. Diameter of membrane 12 cm., walls permit maximum excursion of 1 cm. in each direction. Inside dimensions of box above (shown in longitudinal section) 3 x 3 x 9 cm. The lid is of glass, removable but sealed by modelling clay. *T*, outlet to intrapleural space; *C*, outlet to cardiometer.

C. Response of tambour (above) to moving piston of syringe (below) upper chamber open to outside air. Time, seconds.

D. Response of tambour (below) to moving piston of syringe (above). The upper chamber was connected to the intrapleural space of a dog breathing naturally. The middle record is of intrapleural pressure.

E. From above down: respiratory displacement of tambour, volume scale shown at right; intrapleural pressure, reduced by about 8 cm. H_2O at each inspiration; arterial pressure; time, seconds. The lower chamber of the tambour was connected to a flask with a fixed air space of 100 cc.

membrane. When the upper chamber is first connected to the thorax, air is withdrawn through C_1 to inflate the lungs; but enough should be left to maintain a small pneumothorax at all times, allowing free movement

of air to equalize pressures between the tambour and the intrapleural space. As long as this condition is observed the ventricles fill, during diastole, against the existing intrapleural pressure, plus the slight resistance of the membrane itself. The membrane is thereby displaced upward, and a corresponding volume of air driven over into the thoracic cavity. This movement is reversed at systole. Ventricular volume changes are recorded as large and rapid excursions, superimposed on slower and much smaller respiratory waves.

Details of the tambour are shown in figure 1B. The ordinary lever arrangement obviously cannot be used for recording, on a kymograph in outside air, the excursions of a membrane with subatmospheric pressure on both sides. The membrane here used carries a light disc supporting a flat vertical shaft, both of thin aluminum. The shaft, projecting into the boxlike superstructure, engages a short lever with a mirror pivoted at its fulcrum. The glass window, transmitting the light beam to and from the mirror, is a convex lens, focusing the beam on a ground-glass plate at a distance of 30 to 40 cm. The excursions, too large for direct projection into the camera, are recorded by a camera with an ordinary lens, placed beyond the ground-glass plate. The bulk of the tambour interferes with the simultaneous projection of other light beams, except from an equally short distance. We therefore had to use, for arterial and venous pressures, manometers of relatively high sensitivity and low frequency. Arterial pressure was recorded from the carotid. A catheter was passed down the external jugular vein to the right auricle. It was connected to a manometer so damped that the rapid pulsations in the cardiac cycle were nearly suppressed, the slower respiratory waves of venous pressure being recorded. The arrangement has the advantage of allowing continuous observation of the moving spots on the ground-glass plate.

We have used in the tambour membranes of varying sensitivity. Each was tested by connecting, to the lower chamber, a water manometer and a large syringe containing 100 cc. of air (a volume approximately equal to the average air space around the ventricles in the cardiometer). The piston of the syringe was then moved slowly over a volume range of 50 cc., 25 cc. in each direction from the original position. Between these limits, the pressure change registered with different membranes was 4 mm. H_2O, for the slack type described by Henderson and Barringer (1913a); 24 mm., for medium-weight rubber dam under considerable tension; and 10 mm., for light dam under tension just sufficient to prevent sagging. The results described below were obtained with all three membranes; but we prefer the third type, which was used for all the graphic records shown in the figures. It offers the minimum resistance to ventricular filling, consistent with stability of the membrane when the tambour is placed on its side. This position is most convenient, since it allows horizontal excursions of the beam for recording on film moving vertically.

All the membranes used operate under such low pressures that they probably cannot follow in detail the rapid changes of ventricular volume (Wiggers and Katz, 1922). They appear to be capable, however, of revealing any gross variations of diastolic or stroke volume. To test this, the lower chamber was again connected to the syringe, and the piston moved at varying rates, up to 270 per minute. To the piston was attached a long lever, with a mirror pivoted at the fulcrum for optical recording. The membrane follows the displacement of the piston with fair accuracy. This is true whether the upper chamber of the tambour is left open to outside air (fig. 1C) or is connected to the intrapleural space of an animal breathing naturally (fig. 1D).

It may be pointed out that in figure 1D the movements of the piston were not synchronized with the cardiac cycle, yet they caused only minute changes of intrapleural pressure. Volume changes in the syringe and tambour must therefore have been compensated by a nearly equal movement of air in and out through the trachea, the lungs acting as a second sensitive membrane in series with that of the tambour. In cardiometric recording, with the arrangement shown in figure 1A, volume changes of the ventricles do not have to be so compensated by equal changes of air volume in the lungs. Each change of ventricular volume is accompanied by an opposite change in the volume of blood held in other thoracic vessels; and this redistribution of blood in the cardiac cycle largely compensates for the movement of air back and forth between tambour and thorax. The air drawn into the trachea at systole, and expelled at diastole, needs only to equal the simultaneous change of blood volume in the thorax as a whole. Calculations of the latter are not in entire agreement (Hamilton, 1930; Blair and Wedd, 1939); but according to the highest estimate it amounts only to a fraction of the stroke output from one ventricle.

Under the conditions diagrammed in figure 1A, the membrane will be displaced to some extent by respiratory movements. This effect is of small magnitude. Assuming the resistance of the membrane to be negligible, the air in the entire system behaves as if it were held in a closed pneumothorax, expanding with inspiration in direct proportion to the reduction of intrathoracic pressure. The total air volume of the system, including the variable space of the cardiometer but not the actual pneumothorax, is approximately 460 cc., of which about 240 cc. is on the cardiometer side of the membrane. Supposing intrapleural pressure to change from 755 mm. Hg at expiration to 750 mm. at inspiration, the displacement of the membrane in each respiratory cycle would be 5/755 x 240, or less than 2 cc. With deeper inspiration the respiratory displacement is proportionally greater, but it is always small in comparison to the stroke output from the two ventricles of a large dog. The respiratory effect may be shown in uncomplicated form by connecting the upper chamber of the

tambour to the intrapleural space in the usual manner, and the lower chamber to a flask with a fixed air space of 100 cc. (fig. 1E).

The respiratory changes of intrapleural pressure are effectively transmitted to the cardiometer. This is shown by the fact that respiration can be recorded by a segment capsule connected either to the upper or to the lower chamber, without appreciable difference in the magnitude of the excursions. Any displacement of the tambour membrane from its plane position implies, of course, a pressure difference on the two sides; but the respiratory displacement involves a pressure difference of the order of 1 mm. H_2O, negligibly small in comparison with the respiratory changes of intrathoracic pressure. Ordinarily we recorded respiration by connecting the segment capsule to the outlet at *R* in figure 1A. It was then possible to shift pressure on the ventricles from intrathoracic to atmospheric, or vice versa, without disturbing the respiratory record. A clamp may be applied at C_2 (fig. 1A) and the clamp at C_1 simultaneously released, opening the upper chamber of the tambour to outside air. This reproduces essentially the conditions existing in the experiments of Eyster and Hicks and of Cahoon, Johnson and Michael, referred to above. For comparison we have in each experiment placed the ventricles alternately under intrathoracic and under atmospheric pressure.

The actual pneumothorax required for proper operation of the system is not enough to embarrass the respiration seriously. Our experiments have been continued for three hours or longer, the animals remaining in good condition without artificial respiration. The dogs used were large, weighing from 14 to 22 kgm. They were anesthetized by barbital-sodium, 0.3 gram per kgm., given intraperitoneally. After tracheotomy, the chest was opened by resecting one rib for the greater part of its length. A cardiometer of suitable size was fitted over the ventricles and the chest closed. Connections to the tambour were provided for by two metal tubes of 9 mm. bore, left in the incision. One led to the cardiometer, the other opened into the intrapleural space.

Results. During complete respiratory standstill, induced by weak central stimulation of one sectioned vagus, diastolic volume and stroke output remain for a time nearly constant (fig. 2A). If the apnea is prolonged, there is a very gradual increase of both, accompanied by a rise of arterial pressure, and, if the second vagus is intact, by a moderate slowing of heart rate.

When the animal is breathing, the level of the entire cardiometer record rises with each inspiration. The rise is due, in part, to inspiratory expansion of air in the system and the resulting upward displacement of the tambour; but this effect is relatively small and does not continue into the expiratory pause (compare fig. 2D, first part, with fig. 1E). When due allowance is made for it, there is still an evident inspiratory increase of

ventricular volume, both at the end of diastole and at the end of systole. There is also an augmentation of stroke output. These effects are minimal

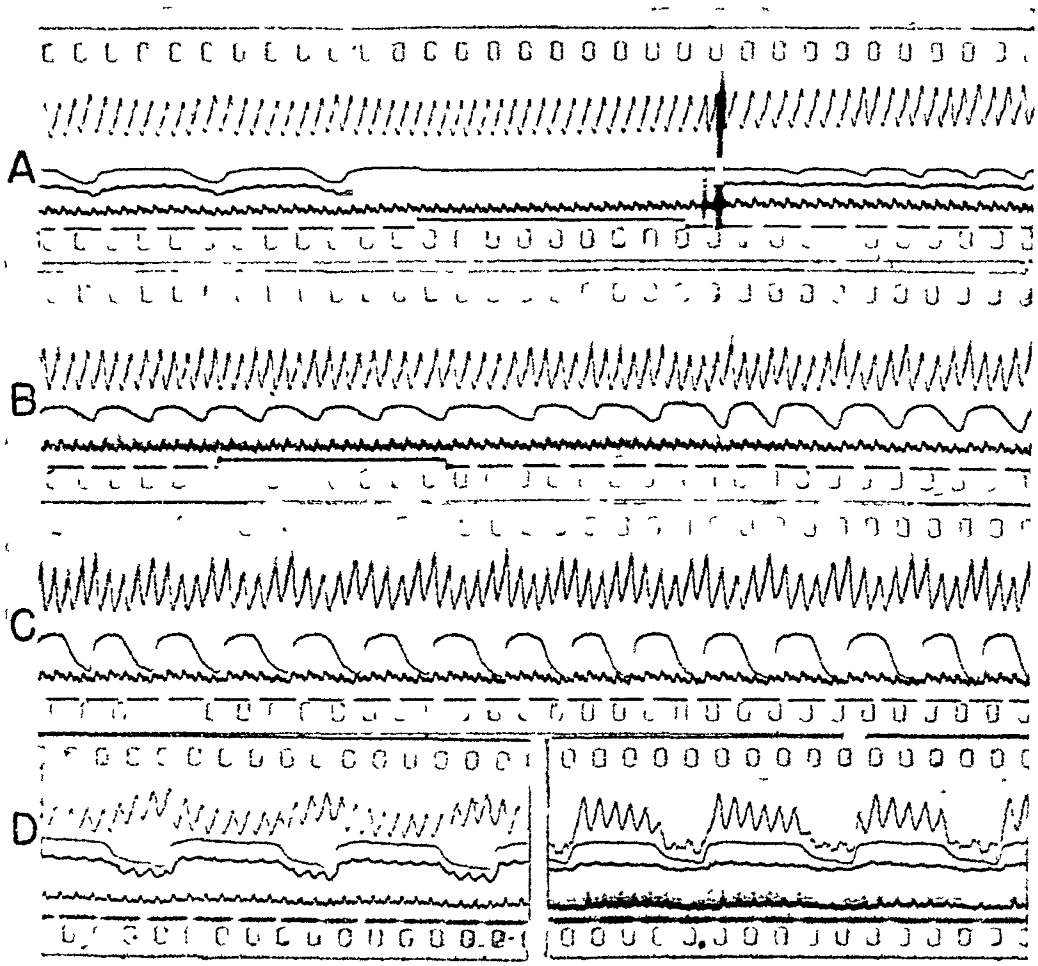

Fig. 2. Each record shows, from above down, ventricular volume, respiration (intrapleural pressure), arterial pressure, and time in seconds. In A and D venous pressure is recorded below respiration. In A, B and C the ventricles were under intrathoracic pressure throughout.

A. Normal breathing, followed by respiratory standstill from weak central stimulation of the right vagus (signal). This in turn is followed by rapid and shallow breathing.

B. Respiration initially rapid and relatively shallow. At the signal, the trachea was partially obstructed by a clamp.

C. Begins 15 seconds after the end of B, the tracheal clamp being left in place. Note effects of deeper inspiration on diastolic and stroke volume.

D. Both vagi sectioned. Ventricles under intrathoracic pressure (first part) and under atmospheric pressure (second part). Note reversal of inspiratory effects on diastolic and stroke volume.

when the breathing is shallow, more marked in ordinary quiet breathing (fig. 2A). They appear in exaggerated degree when inspiration is deepened

and prolonged, either by obstruction of the trachea (fig. 2, B and C) or by section of both vagi (fig. 2D, part 1).

Diastolic volume is maximum at the end of inspiration. With expiration it falls, but may require three or four beats to reach a steady minimum level. Stroke output falls simultaneously, and remains constant during the latter part of a prolonged expiratory pause (fig. 2D, part 1). At this time stroke output is near its minimum, but it becomes still smaller if the onset of the next inspiration happens to coincide with the beginning of systole. The sudden lowering of intrathoracic pressure appears to impede somewhat the emptying of the ventricles. This factor, if it continues to operate throughout inspiration, is more than offset in the later stages by some opposing influence, presumably the increase of diastolic volume. Stroke output reaches its maximum either at the end of inspiration or with the first systole following the onset of expiration. The largest systolic excursions are registered when expiration and systole begin simultaneously. Such a systole starts from maximum diastolic volume, and leaves a residual volume smaller than was left at the end of the preceding systole. The emptying of the ventricles thus appears to be aided by a coincident rise of intrathoracic pressure. Several examples illustrating the behavior described may be found in figure 2. In some animals inspiration augments stroke output nearly as much as diastolic volume (fig. 2, B and C); in others, the increase of stroke output is smaller in proportion, and the volume left at the end of systole relatively large (fig. 2D, part 1). These individual differences may depend on the condition of the heart, or on the effective venous pressure.

The respiratory effects described above appear only when the ventricles are under intrathoracic pressure. If the upper chamber of the tambour is opened to outside air, and the ventricles thereby put under atmospheric pressure, their volume and output diminish with inspiration. This confirms the observations of Cahoon, Johnson and Michael (1940), made under similar conditions. Eyster and Hicks (1933) also noted an inspiratory reduction of stroke output, but a simultaneous increase of diastolic volume. We are unable to account for the discrepancy between the latter finding and our own.

The reversal of the inspiratory effect, when the ventricles are placed under atmospheric pressure, seems due entirely to the abnormally high and unchanging resistance against which they must fill. Since the other thoracic vessels are left under subatmospheric pressure, there is a backward gradient of external pressures, tending to dam blood back into the auricles and veins. This back pressure obviously would increase during inspiration. Under these conditions right auricular pressure, measured against atmospheric, remains nearly constant throughout the respiratory cycle (fig. 2D, part 2). Since external pressure on the auricles and veins is

reduced during inspiration, it is difficult to see how their internal pressure could be held constant, except through distention by an increased volume of blood. Normally, right auricular pressure is well known to fall during inspiration (fig. 2D, part 1). It may also be noted that the respiratory variations of stroke output are much greater under atmospheric than under intrathoracic pressure. In the former condition, the inspiratory reduction of output is accompanied by a marked weakening of the carotid pulse, which may be almost completely suppressed by a deep inspiration (fig. 2D, part 2). Normally, the carotid pulse shows relatively small variations in the respiratory cycle.

Discussion. Since the cardiometer record shows only the combined volume of the two ventricles, other data must be used to distinguish volume changes on the right from those on the left side. From the differential pressure studies of Wiggers (1914) and of Hamilton, Woodbury and Vogt (1939), one would expect inspiration, particularly if forced, to augment filling and output of the right ventricle. Its effect on the left is much less clear. We have invariably found, with deep and prolonged inspiration (vagi sectioned) a fall of pressure in the carotid artery. The fall is greatest when the ventricles are under atmospheric pressure, and under those conditions evidently is due, in part or entirely, to a reduction of output from the left ventricle. When the ventricles are under intrathoracic pressure the inspiratory fall of carotid pressure is less profound but is still present. It might be attributed either to *a*, reduced left ventricular output, masked on the cardiometer record by a simultaneous and greater increase of output on the right side; or to *b*, inspiratory lowering of external pressure on the arteries in the thorax, reducing the total resistance against which the left ventricle empties. The latter explanation seems to imply an increased capacity of the thoracic arteries, and an inspiratory reduction of flow through arteries outside. It is difficult to reconcile either *a* or *b* with the observation of Machella (1936), that blood flow through the femoral artery is augmented during inspiration.

The present paper has dealt only with the variations of ventricular filling and output in the individual respiratory cycle, and not with the influence of respiratory movements on the mean cardiac output over longer periods of time. We realize that in the conscious human subject, under varying conditions of posture and muscular activity, the circulatory effects of inspiration and of expiration might differ somewhat from those observed on the anesthetized dog in the supine position.

SUMMARY

1. A method of cardiometric recording, designed for use with the chest closed, is described. It maintains on the ventricles an external pressure which is always approximately equal to intrathoracic pressure, varying

in the normal manner with the phases of respiration. It can be alternatively used in such a way that the ventricles are left under constant atmospheric pressure.

2. With the ventricles under atmospheric pressure, diastolic volume and stroke output diminish markedly with inspiration (confirming earlier investigators). These are abnormal effects, due to the artificially high resistance against which the ventricles are filled.

3. With the ventricles under intrathoracic pressure, combined diastolic volume and stroke output of the two ventricles are augmented with inspiration. These effects are most pronounced when inspiration is deep and prolonged. They are relatively small in quiet eupneic breathing, and are minimal when the breathing is rapid and shallow.

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THE EXTENT TO WHICH RADIOACTIVE CHLORIDE PENETRATES TISSUES, AND ITS SIGNIFICANCE

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In a preceding publication Manery and Bale (6) reported the results of a survey of the extent and rate of penetration of injected radioactive sodium (Na^{24}) into mammalian tissues. There was some variation in the rate at which Na^{24} penetrated the tissues from the plasma, but in every case penetration proceeded until the tissue:plasma ratio of the radioactive isotope was the same as that for the normal sodium isotope. This value was never exceeded and therefore penetration was considered complete when equality of the ratios was attained. In many tissues entrance was complete in about 8 minutes and this finding, with some reservations (see (6)), was taken to indicate that all of the sodium contained in such tissues was extracellular. Na^{24} entered other organs more slowly, complete penetration requiring several hours. In some instances the presence of a small amount of intracellular sodium was suggested, but in other cases the slow rate of entrance could be attributed to other causes.

Since sodium and chloride differ so markedly in concentration in many tissues it seemed desirable to make a similar survey of the extent and rate of penetration of the radioactive isotope of chlorine (Cl^{38}). This was administered as the chloride of lithium. The isotope used disintegrates so rapidly that the information which can be obtained is limited. However, although the variation is fairly large, the data seem to show that radioactive chloride likewise becomes distributed from the plasma into most tissues until its tissue:plasma ratio is the same as that for normal chloride chemically determined. There is more evidence of intracellular chloride in these data than there was of intracellular sodium in the data referred to above. A comparison of the behaviour of the two unstable isotopes allows a more reasonable measurement of the extracellular water of many tissues than has hitherto been possible. An abstract of the experiments has already been published (5). A more detailed account will be presented here.

METHODS AND CALCULATIONS. Radioactive chloride ($_{17}Cl^{38}$)[1] of half

[1] The radioactive salt was kindly prepared for us by Dr. S. N. Van Voorhis and Dr. C. W. Strain of the Department of Physics, operating under a grant from the Rockefeller Foundation. We are particularly indebted to them for their excellent coöperation during these experiments.

life 37 minutes (8) was administered in a 1 per cent solution of lithium chloride. Five rats were injected intraperitoneally with 2.1 ml. per 100 grams of body weight and 5 rabbits intravenously via an ear vein with 2.8 ml. per kgm. of body weight. Although the half life of this chloride isotope is short, the activity was sufficiently high (5 millicuries per liter) to permit experiments of 5 to 6 hours' duration. During this period the salt was dissolved, the solution injected into the animal and time allowed for its distribution. The animal was then decapitated and bled. The tissues were dissected, ashed, subjected to the chemical procedures described below, and the radioactivity of the final solution determined with a Geiger-Müller counter. In some experiments two counters were used. The methods of dissecting the tissues, of determining chloride chemically, and of counting the solutions, were essentially the same as those described by Manery and Bale (6). Separate samples were taken for the determination of radioactivity and of chloride by the chemical method.

The lithium component of the salt also becomes radioactive during deuteron bombardment, but since the half-life of the lithium isotope is only 0.88 second its activity is soon dissipated. Although a reagent grade of lithium chloride was bombarded for these experiments it was found to contain a trace of an element other than chlorine or lithium which became radioactive. Sodium seemed to be the most likely contaminant. Hence the chloride of the solution was precipitated as silver chloride in the presence of an excess of sodium. The precipitate was dissolved and the final solution showed a true decay curve of an isotope the half life of which was 37 minutes. This solution was used as a standard of comparison for the unknown solutions.

Similarly, the chloride of each sample of tissue or body fluid was precipitated as the silver salt and the radioactivity of a solution of the precipitate determined. The samples were placed in 50 ml. centrifuge tubes containing sodium nitrate which was used to decrease the possibility of radioactive sodium being carried down in the silver chloride precipitate. Silver nitrate and nitric acid were then added as in most chloride methods in common use. The tubes were heated on a steam bath until the supernatant solution was clear, which required 1 to 2 hours. They were subsequently centrifuged and cooled on ice to solidify the fat. The supernatant liquid was removed by suction, care being taken not to remove any of the fat globules. After washing the precipitate twice with distilled water it was dissolved in 2 to 20 ml. of 1 per cent solution of potassium cyanide. The fat present was likewise dissolved. Vigorous stirring and slight warming were sometimes necessary to dissolve the larger amounts of precipitate. This solution was counted directly or diluted as desired. Although a solution of potassium cyanide was employed in all experiments reported here, ammonium hydroxide seems to be equally useful for dissolving the precipitate.

The half-life of Cl^{38} is so short that its disintegration is perceptible during the 4 minute periods of counting. Hence each tissue was counted for 2 to 5 periods carefully timed and the results for each period calculated from the theoretical decay curve. The averages of all the periods for each tissue are the figures presented in the tables. All values except those asterisked were calculated from counts which were many times the background count. Those which were twice the background or less were discarded. The greatest percentage differences between members in a series of 16 duplicate, triplicate or quadruplicate samples were 27 and 29 per cent while the average including these values was 11 per cent and excluding them was 8.5 per cent.

The methods of calculation and presentation of the data have been explained in the previous paper (6) where the precise meanings of the symbols used are given. It will suffice to state that a value called $(H_2O)_E$ or the extracellular water was calculated as follows in grams per 100 grams of fresh tissue:

$$(H_2O)_E Cl^{38} = \frac{\text{tissue counts per min. per kgm.}}{\text{plasma counts per min. per liter}} \times 0.95 \times 0.93 \times 100$$

These values were compared to similar values calculated from the chemically determined chloride.

Results. It should be pointed out at the outset that no toxic effects of lithium chloride were observable in these acute experiments. Even after intravenous injection into rabbits there were no outward manifestations of abnormal behaviour in 52 minutes. A comparison of the chloride concentrations of tissues reported here with those in 2 former papers (6, 7) shows that the electrolyte pattern was not appreciably altered by the treatment.

Essentially only two periods of time were studied, a short period of a few minutes which was shown (6) to be adequate for radioactive sodium to enter the extracellular phase of tissues, and a longer period ranging from one-half to one hour which is sufficient for equilibrium to be established between plasma and most tissues. Penetration was taken to be complete when the value of $(H_2O)_E$ calculated from Cl^{38} became equal to that calculated from Cl. The results for 5 rats are shown in table 1 and for 5 rabbits in table 2. To conserve time the tissues were not always analyzed chemically for chloride. By consulting the data in references 6 and 7 it can be seen that the variation in tissue chloride between stock animals prepared in this manner is small.

In many of the tissues listed in both tables the Cl^{38} values closely approximate the Cl values. As in the radioactive sodium experiments the tissues can be divided into two groups, those in which the Cl and Cl^{38} values are equal indicating that the penetration of radioactive chloride into

the Cl-containing phase is completed rapidly, and those in which the Cl value exceeds the Cl^{38} value in all of the periods of time studied. Since one hour was the longest period allowed before decapitation, we have no data to show that the Cl^{38} slowly penetrated the entire Cl-containing phase of tissues into which it was delayed, as was the case with radioactive sodium.

TABLE 1

Data obtained from rats injected intraperitoneally with lithium chloride containing radioactive chloride

TISSUE	RAT 1		RAT 2	RAT 3		RAT 4	RAT 5
	Rat weight (grams):						
	294		264	334		280	284
	8 min.		8 min.	28 min.		30 min.	32 min.
	Values of $(H_2O)_E$ (grams per 100 grams fresh tissue) calculated from						
	Cl	Cl^{38}	Cl^{38}	Cl	Cl^{38}	Cl^{38}	Cl^{38}
Skin	38.3	36		48.6		38	32*
Kidney	48.9	126 (?)		48.6	74 (?)	41	38*
Liver	26.4	97 (?)	52 (?)	27.7	37 (?)	24*	
Testes	54.2	80 (?)	27	53.8	27	22	16*
Gastrocnemius	12.4	13	7.8	14.8	13		12*
Heart	23.4		21		22		22*
Brain	27.4	4.2*	2.6	29.3		6.0	negl.
Plasma Cl (m.eq. per l.)	102.8			103.9			
Per cent distribution			32	29		28*	25
$\frac{Cl^{38} \text{ perit. fl.}}{Cl^{38}\ (H_2O)_E}$			8.1	4.1		4.6*	3.9
Per cent dose in plasma	3.3		3.1	7.0		7.2	8.2

Negl. = negligible.

* Values asterisked were calculated from counts which were only 2 to 4 times the background count.

(?) No significance is attached to the fact that these figures exceed the Cl values, since they are not substantiated by the data on rabbit tissues. It seems likely that contamination with peritoneal fluid occurred in spite of efforts to avoid it. A pool of peritoneal fluid was always evident and its radioactivity, as the table shows, is 4 to 8 times that of plasma.

Generally speaking, the first group contains skin, kidney, liver, gastrocnemius, heart, abdominal muscle and tendon. The tissues of the second group, which show a great difference between the Cl and Cl^{38} values, are testes, pyloric mucosa and brain.

In considering the tissues of the first group it can be seen that, with the exception of figures followed by a question mark in table 1, the Cl and Cl^{38} values are similar except for the kidney of rabbit 4. The skin of rab-

bits (table 2) possesses more chloride than that of rats but its entire Cl phase seems to be quickly penetrated. Although the Cl^{38} and Cl values approximate each other in kidney cortex it is likely that the tubules contain intracellular chloride. Reference has been made to this point with regard to the rapid entrance of Na^{24} into the Na phase of kidney (6).

TABLE 2

Data obtained from rabbits injected intravenously with lithium chloride containing radioactive chloride

TISSUE	RABBIT 1		RABBIT 2		RABBIT 3		RABBIT 4		RABBIT 5
	Rabbit weight (kgm.):								
	2.6		2.9		3.0		2.9		2.7
	11 min.		41 min.		45 min.		48 min.		52 min.
	Values of $(H_2O)_E$ (grams per 100 grams fresh tissue) calculated from								
	Cl	Cl^{38}	Cl	Cl^{38}	Cl	Cl^{38}	Cl	Cl^{38}	Cl^{38}
Skin	60.6	56				53	62.6		61
Kidney	38.8	41					45.4	59	41*
Liver	19.3	18	19.8	16		17*	21.4	25*	16
Testes		28				24	45.4		46*
Gastrocnemius	11.6	8.3*	11.5	8.4	12.3		11.4	9.4*	9.0
Heart	29.6	31	30.2	29	28.8				28*
Abdominal muscle	15.8	14*	16.9	14	15.9				18
Pyloric muscle	40.4	30	44.6	40	38.3	26*	43.8		39
Pyloric mucosa		33			52.4	34*	58.9	38	30
Ear cartilage	50.2	37*					48.5	58*	62
Tendon	57.1	56			58.4			66	59
Brain	31.8	4.7*				10	33.0	11*	9.5
Plasma Cl (m.eq. per l.)	105.2		98.8		105.1		101.5		
Per cent distribution	20		17		26		17		23
Per cent dose in plasma	13		12		9		13		10

* Values asterisked were calculated from counts which were only 2 to 4 times the background count.

In the heart, abdominal, gastrocnemius and pyloric muscles the $(H_2O)_E$ values calculated from Cl^{38} simulate those calculated from Cl. However, because there was some suggestion in the Na^{24} data (see table 2 of reference 6) that Na^{24} did not enter the entire Na phase of muscle in 20 minutes, it should be pointed out that in ten corresponding pairs the Cl^{38} value is lower than the Cl value by an average of 18 per cent. In view of the large percentage difference between duplicate analyses (7 and 11 per cent for Na^{24} and Cl^{38} respectively) these two sets of data scarcely support, but

certainly do not disprove, the proposal recently made for frog muscle by Conway et al. (3) that a small part of the total sodium and chloride of muscle might be intracellular.

The data on cartilage and tendon as they stand (table 2) indicate that the entire Cl phase is freely accessible to Cl^{38}. It should be recalled that tendon is dense connective tissue with few cells and that cartilage is outstanding because the sodium concentration is greatly in excess of the chloride concentration. Cl^{38} is not appreciably delayed in its entrance into the Cl phase of cartilage and it seems reasonable to consider that it represents the extracellular phase of the tissue. The extracellular water of rabbit ear cartilage when calculated from chloride is only slightly less than the total water of the tissue as found by Manery and Hastings (7). This leaves half the sodium content in a solid non-aqueous phase.

Of greater interest perhaps are tissues of the second group. Relatively small amounts of radioactive chloride are found in brain tissue even in one hour after injection. Judging from previous estimations of the hemoglobin content of brain (7) this is probably somewhat more than would be contained in the blood of the tissue but still is so small that only one third of the total chloride phase has been penetrated. Other instances of the slow penetration of substances into brain have been cited (6) and will not be repeated here. As will appear in the discussion these data cannot yet be taken to indicate intracellular chloride.

Testes and pyloric mucosa are exceptional because the chloride content is greatly in excess of the sodium, making the Cl phase much larger than the Na phase. In testes only one-half of the Cl phase is penetrated by Cl^{38} in 45 minutes. The differences are large and consistent except for rabbit 5. The average value of $(H_2O)_E$ for both types of animals calculated from Cl^{38} is about 28 while that calculated from Cl is nearly 50. The Na value, on the other hand, is 30 (6, 7) for both rats and rabbits, which is almost the same as the Cl^{38} value. Radioactive chloride, then, has penetrated a volume equal in magnitude to the Na phase in the periods of time studied. In our previous report data on Na^{24} indicated that all but 20 per cent of the entire Na phase was entered rapidly by Na^{24}. It is possible that only this portion is penetrated by Cl^{38}.

In pyloric mucosa a similar situation exists. The value of $(H_2O)_E$ calculated from Cl^{38} amounts to slightly more than half that calculated from Cl and is almost equal to the Na value. In this tissue, too, the figures indicate that Cl^{38} has entered a volume equal to the Na phase and has not progressed beyond it in 52 minutes. Whether the "excess" chloride (i.e., chloride in excess of sodium) found by analyses in stomach mucosa occurs within the secreting cells or in the lumen of the glands rather than in supporting cells is not yet known, but in any case plasma chloride does

not seem to freely diffuse into this volume or to exchange with the chloride already there.

It seems improbable that the similarity of the Cl^{38} values to the Na values in these tissues is mere coincidence. A simple interpretation of the finding is to consider that all of the sodium found by analysis is contained in the extracellular phase. Into this phase Cl^{38} promptly enters, but it cannot exchange so rapidly with the remaining chloride, of the pyloric mucosa for example, because this portion is either intracellular or in the gland lumens. The rate of entrance into the second phase might depend on secretory processes which in this case were slower than diffusion. A similar interpretation might apply to testes. If this interpretation can be accepted the extracellular water of both testes and pyloric mucosa would measure about 30 per cent of the wet weight. A more complete study of the rate of entrance into the entire Cl space would be desirable in order to demonstrate the existence of two phases entered at different rates.

By way of summary a graphic comparison of representatives of the two groups of tissues discussed above has been made in figure 1.

The water of the body into which the absorbed Cl^{38} became distributed can be calculated by assuming the same concentration in the water as in a plasma ultrafiltrate. This was called the per cent distribution (see equation 5 (6) for the exact calculation) and may be taken as a good approximation to the true volume of extracellular water. It was found to be about 29 per cent of the body weight in rats (table 1) and about 21 per cent in rabbits (table 2). The results are more variable but are in fairly close agreement with similar values calculated for radioactive sodium. The urine was collected in each of the rabbit experiments and analyzed in order to ascertain the amount of Cl^{38} excreted. It was found to contain so few counts that collection and analyses of urine were omitted in the rat experiments.

The ratio of Cl in peritoneal fluid:Cl^{38} in $(H_2O)_E$ for rats (table 1) indicates that more time was required for equilibrium to be established in the case of lithium chloride than was observed when sodium chloride was injected (table 2 of (6)).

The rate of disappearance of radioactive chloride from the plasma of rabbits was likewise studied. Blood was collected from veins of the ear on the opposite side to that which received the injection, and was centrifuged at once. In the 3 cases where collections were made in 5 to 8 minutes after injection only about 15 per cent of the dose injected remained in the plasma. In the subsequent 40 minutes this dropped only a few per cent. The values obtained at decapitation are given in the tables. Again the isotopes afford an illustration of the rapidity with which diffusion occurs from the plasma into the extracellular fluid of the body. It should be

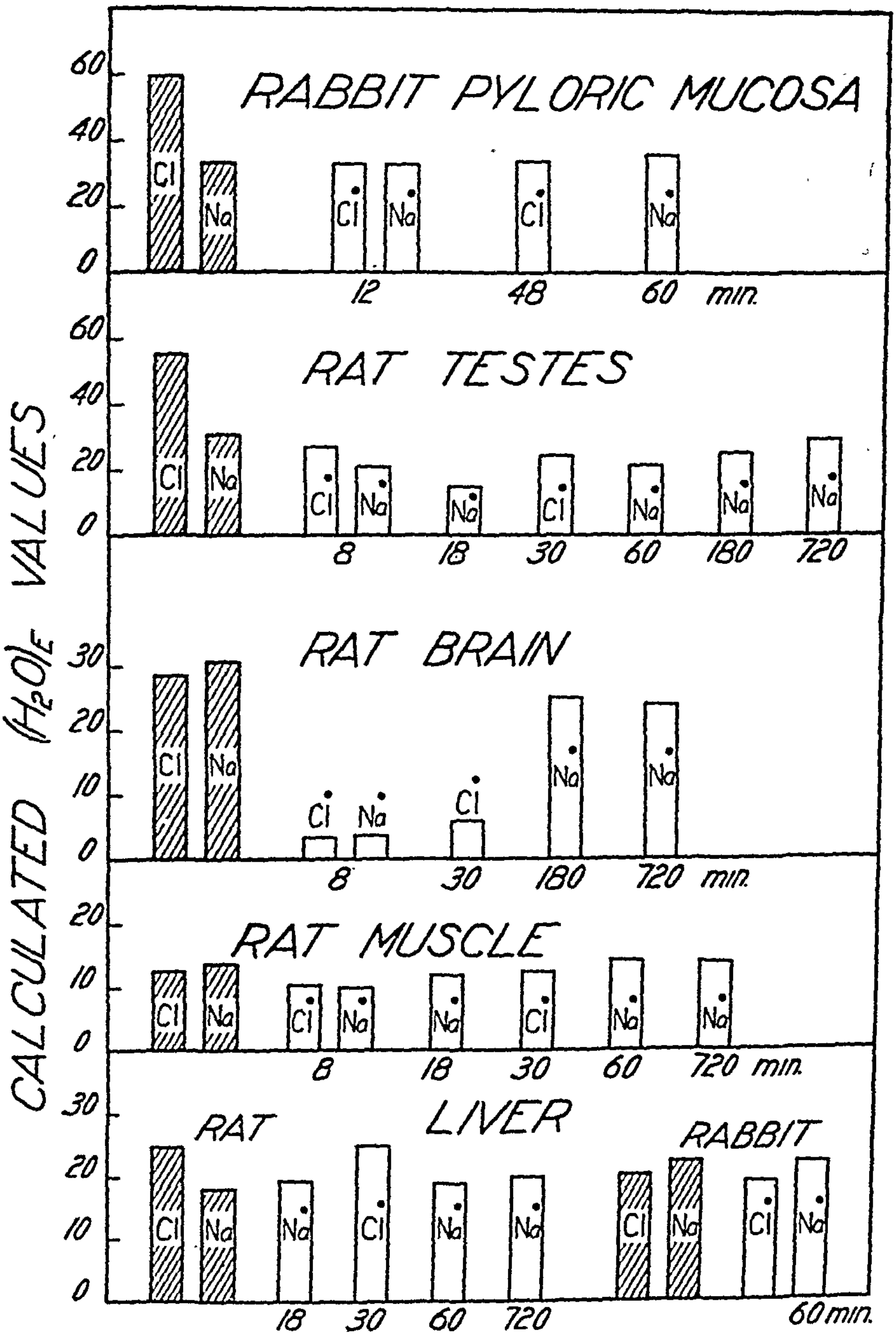

Fig. 1. A comparison of the values of $(H_2O)\hat{E}$ calculated from radioactive chloride (Cl•) with those calculated from radioactive sodium (Na•).

Values of $(H_2O)\hat{E}$ are calculated from Na and Cl chemically determined (cross hatched blocks), and from the radioactivity of the isotopes Na^{24} and Cl^{38}, according to the equation given in "methods and calculations." These values in grams per 100 grams of fresh tissue for sodium probably closely approximate the extracellular water of each tissue. The figures on the horizontal axis refer to the period of time which elapsed between injection of the radioactive solution and decapitation. The radioactive Na values (Na•) were taken from Manery and Bale (6).

recalled (see equation 6 of reference (6)) that the actual plasma volume was not measured for these calculations. The blood volume was assumed to be constant. Hematocrit values were determined in the rabbit experiments but an average value was assumed in the case of rats.

DISCUSSION. It was shown by Manery and Hastings (7) that mammalian soft tissues could be grouped into *a*, those in which the Na:Cl ratios were the same as in an ultrafiltrate of plasma, and *b*, those which contained chloride in excess of sodium. In other words the values of $(H_2O)_ECl$ equal those of $(H_2O)_ENa$ in group *a* but $(H_2O)_ECl$ is always greater than $(H_2O)_ENa$ in group *b*. To a group *c* we might relegate skeletal structures in which $(H_2O)_ENa$ greatly exceeds $(H_2O)_ECl$. A further characterization of these groups is now possible. Radioactive isotopes of sodium and chloride penetrate all of the tissues of group *a* (except brain) *with great rapidity, becoming distributed between the tissue and the plasma* to the same magnitude as the normal isotope. Neglecting minor differences the tissues thus characterized are skin, kidney, liver, muscles and tendon. In group *b*, where the Cl phase exceeds the Na phase in magnitude radioactive sodium enters the entire Na phase fairly rapidly but even in one hour radioactive chloride has penetrated only the Na phase and is definitely delayed in entering the entire Cl phase. Testes and pyloric mucosa are the outstanding members of this group. Our data on the rates of entrance of the radioactive isotopes into tissues of group *c* are rather incomplete but, since these are highly specialized, they require a rather different type of laboratory handling and of theoretical consideration.

The data obtained using radioactive sodium and chloride, if interpreted as indicated above, lend some support to the hypothesis of Manery and Hastings that there are three chemical phases in tissues: 1, the extracellular phase, $(E)_p$, which is in ionic (Donnan) equilibrium with plasma; 2, an intracellular phase, $(C)_1$, which contains neither Na nor Cl; and 3, an intracellular phase, $(C)_2$, which contains Cl but not its equivalent of Na. Furthermore, a method using these two isotopes is suggested for measuring the extracellular water of tissues where the method based on the analyses of the total Na and Cl failed, and for fractionating the Na and Cl of a tissue so that a measure of the intracellular portion of sodium and chloride can be attained.

It should be reiterated that these properties of tissues have been stated in the most general terms and that each tissue requires particular consideration. Brain, for example, contains sodium and chloride in ultrafiltrate proportions which suggests that the Na and Cl exist in the extracellular phase of the tissue. However neither radioactive sodium nor chloride enter the NaCl-containing phase of brain for long periods of time after administration, and in this regard they do not differ from phosphate which is usually intracelllular. Before the slow penetration can be taken to

indicate intracellular NaCl other factors which might prevent free diffusion into the extracellular phase of the tissue must be considered. It is difficult to ascribe this difference in the rate of penetration between brain and other tissues of group *a* to the number and distribution of capillaries, although the white matter is considered by Cobb and Talbott (2) to contain fewer capillaries than grey matter or resting muscle. It is this portion of the blood-brain barrier, namely, that located in the region of the cerebral capillaries which concerns us, and not the barrier at the chorioid plexuses. Wallace and Brodie (9) have likewise been interested recently in this portion of the barrier.

Histological differences between cerebral capillaries and those elsewhere, which could give rise to different permeability properties, have not been clearly demonstrated. It has been known however that certain dyes, for example trypan blue (see (4) for review) will not pass through cerebral capillary walls although they easily traverse those of other tissues. It would be surprising if ions like sodium, chloride and phosphate were delayed at the cerebral capillary walls from entering brain extracellular space. Real differences seem to occur between brain and other tissues in the relation of the connective tissue elements to the capillary walls and to nerve cells (Cobb, 1). Perhaps diffusion through the extracellular space is slowed by virtue of this particular arrangement. Until more information is available, therefore, we do not know whether the mode of penetration of radioactive sodium and chloride into brain is indicative of intracellular sodium and chloride or is due to some other factor.

SUMMARY

The extent and rate of penetration of Cl^{38} into rat and rabbit tissues were measured by comparing the ratio of the tissue:plasma concentration of Cl^{38} to a similar ratio for Cl chemically determined. When the two ratios were equal penetration was complete.

Penetration was completed in a few minutes after injection in kidney, liver, muscles, cartilage and tendon. Cl^{38} did not enter the entire Cl-containing phase of testes and pyloric mucosa even in one hour but penetrated a volume of tissue equal in magnitude to the Na phase. Only a trace of the radioactive isotope was found in brain.

The significance of these findings is discussed.

We wish to express our indebtedness to Dr. W. F. Bale of the Department of Radiology for supervising the operation of our Geiger-Müller counter, and for assisting with a second counter in some of the experiments.

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"ALKALINE" PHOSPHATASE ACTIVITY OF THE PROXIMAL CONVOLUTED TUBULES AND THE MECHANISM OF PHLORIZIN GLYCURESIS

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It is generally accepted that the principal action of phlorizin is upon the kidneys (1, 2), where reabsorption of glucose from the glomerular filtrate by the cells of the proximal convoluted tubules is inhibited (3, 4). The crux of the problem of phlorizin glycuresis now appears to lie in determining what specific cellular mechanisms are involved in the "active" processes of tubular reabsorption and what phase of these processes is interrupted by phlorizin.

In 1933 Lundsgaard suggested (5) that "the 'active' step in tubular reabsorption of glucose consists of phosphorylation [which he associated with the large amounts of phosphatase present in kidney tissue] and that the effect of phlorizin is to block phosphorylation." In support of this view, Lundsgaard reported (5) that phlorizin in M/200 to M/50 concentration definitely inhibits both dephosphorylation of glycerophosphate by renal extracts and the formation of phosphoric esters in muscle pulp.

The attempt to relate phosphorylation of glucose to the synthesizing action of renal phosphatase soon led to difficulties and many questioned the validity of this whole concept of glucose reabsorption and phlorizin glycuresis: 1. Lundsgaard himself subsequently found (6) that the dosage of phlorizin necessary for maximal glycuresis in perfused pump-lung-kidney preparations was only about $\frac{1}{5}$ that required for inhibition of esterification in muscle pulp. This discrepancy later appeared to be explained by the observation (7) that phlorizin following injection is concentrated in the proximal convoluted tubules where glucose reabsorption takes place; but whether it is sufficiently concentrated to inhibit renal phosphatase was not determined. 2. Lambrechts found (7) no parallelism between the glycuretic action and the in vitro inhibiting effect on renal phosphatase of various substances related to phlorizin. Phloroglucin and salicin, which are not glycuretics, slightly inhibited renal phosphatase activity in vitro whereas arbutin and phlorin caused glycosuria but did not inhibit the enzyme. 3. The in vitro inhibition of renal phosphatase activity by

phlorizin reported by Lundsgaard (5) was found to be inconsiderable by subsequent investigators (3), particularly in properly buffered solutions (8). 4. No consistent difference was observed between the phosphatase activities of renal extracts of phlorizinized and normal rats (3).

These discrepancies led Walker and Hudson (3) and others to infer that phlorizin does not cause glycuresis by inhibiting renal phosphatase, a conclusion which is supported by our results. We have employed the Gomori-Takamatsu histochemical technique (9, 10) for demonstrating phosphatase in tissues. This method permits of precise cellular localization of the enzyme and also gives a rough measure of the amount of enzyme present.

It can be shown in this way that the proximal convoluted tubules are extremely rich in "alkaline" phosphatase (9–12), which is largely concentrated at or near the luminal border of the cells. We found no indication either by this direct histochemical approach or by conventional chemical methods of any significant inhibition of the renal phosphatase activity of the proximal convoluted tubules in maximally phlorizinized animals as compared with normal controls. This result was obtained in both acutely and chronically poisoned rats and following direct injection of phlorizin into one renal artery of the dog by the Zuntz technique (1).

Procedure. 1. *Acute phlorizin poisoning.* Sixteen albino rats weighing about 200 grams were injected intraperitoneally with 10 mgm. phlorizin (recrystallized) in 2 cc. of 2 per cent sodium bicarbonate. Fifteen minutes after injection a urine sample for sugar determination was obtained from the first rat, which was then sacrificed. This procedure was repeated at intervals up to 90 minutes after injection. A part of each kidney was fixed immediately for histochemical examination. Another portion was weighed, ground, extracted with water and the "alkaline" phosphatase activity of the extract determined by the King and Armstrong method (13). Unincubated aliquots of the aqueous extracts gave control readings. The results are expressed in units = number of milligrams phenol liberated per hour. Kidney sections from 4 untreated rats served as controls.

2. *Chronic phlorizin poisoning.* Three albino rats weighing about 200 grams were given daily subcutaneous injections of 20 mgm. phlorizin in 2 cc. peanut oil for 5 days. Quantitative sugar analyses were made on measured 24 hour urine samples. The kidneys were studied histologically and chemically by the methods indicated.

3. *Zuntz experiments.* After sodium pentobarbital anesthesia and preliminary saline infusion (300 cc.), the kidneys, renal arteries and ureters of three dogs were exposed by posterior approach. No. 6 French ureteral catheters were then tied in place. When approximately equal urinary flow from both kidneys was established, control urine samples were collected for sugar and non-protein nitrogen determinations and small biopsies

from each cortex were obtained. Hemorrhage was controlled by cautery. Phlorizin was then rapidly injected into the left renal artery in a dosage of 0.5 mgm./kgm. body weight (in 0.5–0.75 cc. 2 per cent sodium bicarbonate). The right artery was temporarily occluded by extrinsic pressure during injection into the left. Specimens of urine and biopsies of the renal cortices were taken immediately after the injection and at intervals thereafter. Sugar and non-protein nitrogen were estimated in the urine samples by the Folin-Wu methods. Histochemical and chemical studies were made of the renal cortex biopsies as described.

The Gomori-Takamatsu technique[1] involves incubation of uniform, thin, mounted sections for 2 hours at 38°C. in a mixture of sodium β-glycerophosphate and calcium nitrate buffered at pH 9.1 with sodium barbital. At the sites where phosphate is liberated from the substrate through the action of the enzyme, a colorless calcium phosphate complex is formed. The sections are then immersed into silver nitrate solution and simultaneously exposed to ultraviolet light. Brown granules appear where the complex has formed as a result of the enzyme activity. Serial sections of phlorizinized kidneys were made to exclude local variations; and to facilitate comparison with controls, the sections were mounted on the same slide. The enzymic activity of the cells of the proximal convoluted tubules could be estimated adequately by the following criteria: intensity of color and size of the granules, the distance between them, and the proportion of the cytoplasm occupied. The activity was graded from 4+ to 1+. By rigid uniformity in the steps of the technique, satisfactorily reproducible results could be obtained.

RESULTS. 1. *Acute phlorizin poisoning* (table 1, fig. 1A, B). Fifteen minutes after injection, when glycuresis was already maximal, the phosphatase activity of the proximal convoluted tubules by the histochemical method was unchanged as compared with the controls. Chemical analyses of the renal cortex however showed a moderate, but consistent and perhaps significant reduction. Thirty minutes after injection, when glycuresis was still maximal, the results by both methods showed no significant decrease. One kidney in 2 animals gave slightly less enzymic activity by the histochemical method. Sixty and 90 minutes after injection, when glycosuria was still maximal, enzymatic activity was normal by both methods. No convincing inhibition of the "alkaline" phosphatase activity of the proximal convoluted tubules was observed in the acute experiments.

2. *Chronic phlorizin poisoning* (table 2). In 2 of the animals in which urinary excretion of sugar was pronounced, averaging 2 grams a day, the chemical and histochemical determinations showed essentially normal

[1] We employed the improved procedure of Kabat and Furth (12) to whom we are greatly indebted for details in advance of publication. Magnesium salts were not added to the substrate mixture.

"alkaline" phosphatase activity. One kidney showed a slight decrease by the histochemical method, of doubtful significance. In the third animal, in which glycuresis was less marked, similar results were obtained by both methods.

TABLE 1

Renal "alkaline" phosphatase activity of rats after acute phlorizin poisoning (intraperitoneal injection of 10 mgm. phlorizin)

RAT NUMBER	MINUTES AFTER INJECTION	URINE QUALITATIVE SUGAR	"ALKALINE" PHOSPHATASE ACTIVITY OF RENAL CORTEX TISSUE: Histochemical			RAT NUMBER	MINUTES AFTER INJECTION	URINE QUALITATIVE SUGAR	"ALKALINE" PHOSPHATASE ACTIVITY OF RENAL CORTEX TISSUE: Histochemical		
			Right kidney	Left kidney	Chemical				Right kidney	Left kidney	Chemical
					units /gram						*units /gram*
1		0	4+	3+		11		4+	4+	4+	
2	Control	0	4+	3+		12		4+	4+	4+	384
3		0	4+	4+	370						
4		0	4+			13		4+	4+	4+	
						14	60	4+	4+	4+	
5		4+	4+	4+	202	15		2+	4+	4+	
6	15	4+	4+	4+	288	16		4+	4+	3+	362
7		4+	4+	4+	312						
8		4+	4+	4+	266	17		4+	3+	4+	
						18	90	3+	3+	4+	
9		3+	2+	3+		19		3+	4+	4+	
10	30	4+	2+	4+		20		4+	4+	4+	422

TABLE 2

Renal "alkaline" phosphatase activity of rats after chronic phlorizin poisoning (five daily subcutaneous injections of 20 mgm. phlorizin)

RAT NUMBER	AVERAGE DAILY URINE SUGAR	PHOSPHATASE ACTIVITY OF RENAL TISSUE: Histochemical		
		Right kidney	Left kidney	Chemical*
	grams			*units/gram*
21	1.9	4+	4+	224
22	2.2	2+	4+	260
23	0.7	4+	4+	326

* Determinations made on cortex and medulla of half a kidney. Average value of 3 normal rat kidneys prepared the same way: 276 units.

3. *Zuntz experiments* (table 3, fig. 1C, D). It was hoped that by injecting the drug into one renal artery the enzyme activity could be studied in kidneys that were excreting different amounts of glucose. We were

unable to take biopsies of the right (non-injected) kidney before sugar appeared in the urine samples of that side. A probably significant differential in sugar excretion was obtained, however, in urine specimens taken 3 minutes after the injection. No convincing decrease in the phosphatase activity of the proximal convoluted tubules was found in the kidneys excreting large amounts of glucose as compared with the same before the injection of phlorizin.

TABLE 3

Representative protocol showing results of injection of phlorizin into the left renal artery of the dog (Zuntz technique) upon the "alkaline" phosphatase activity of the renal cortex of both kidneys

KIDNEY	URINE			TISSUE BIOPSIES OF RENAL CORTEX		
	Minutes after injection collection was begun	Volume	Glucose	Minutes after injection biopsy taken	Phosphatase activity: Histochemical	Phosphatase activity: Chemical
		cc.	mgm. per cent			units/gram
Left	Before injection	2.8	95	Before injection	4+	135
Right	Before injection	2.8	61	Before injection	4+	159
Injection: 8.4 mgm. phlorizin (0.5 mgm./kgm.) into left renal artery						
Left	3	2.6 in 2 min.	980	6	3+	111
Right	3	2.9 in 2 min.	810	8	4+	164
Left	10	3.3 in 1½ min.	2090	15	3+	145
Right	10	3.3 in 1½ min.	1980	13	4+	70*
Left	20	3.8 in 1½ min.	1400	23	3+	63*
Right	20	3.0 in 1½ min.	1453	25	4+	197
Left	30	2.0 in 1½ min.	1760	35	4+	190
Right	30	2.1 in 1½ min.	1620	37	4+	106
Left	60	1.9 in 2 min.	1880	65	4+	
Right	60	1.5 in 2 min.	1880	65	4+	

* Biopsies taken too close to cauterized areas.

In the interpretation of the histochemical results several points had to be considered. 1. Phlorizin might have diffused out of the tissues during fixation by absolute alcohol or a hypothetical inactive phlorizin-phosphatase complex might have been dissociated during fixation and the enzyme reactivated. In fresh tissue fixed only by freezing, no difference could be found in the renal "alkaline" phosphatase activity of normal and phlorizinized rats. 2. During incubation, the water soluble phlorizin might have diffused out leaving a concentration in the tissues too low for

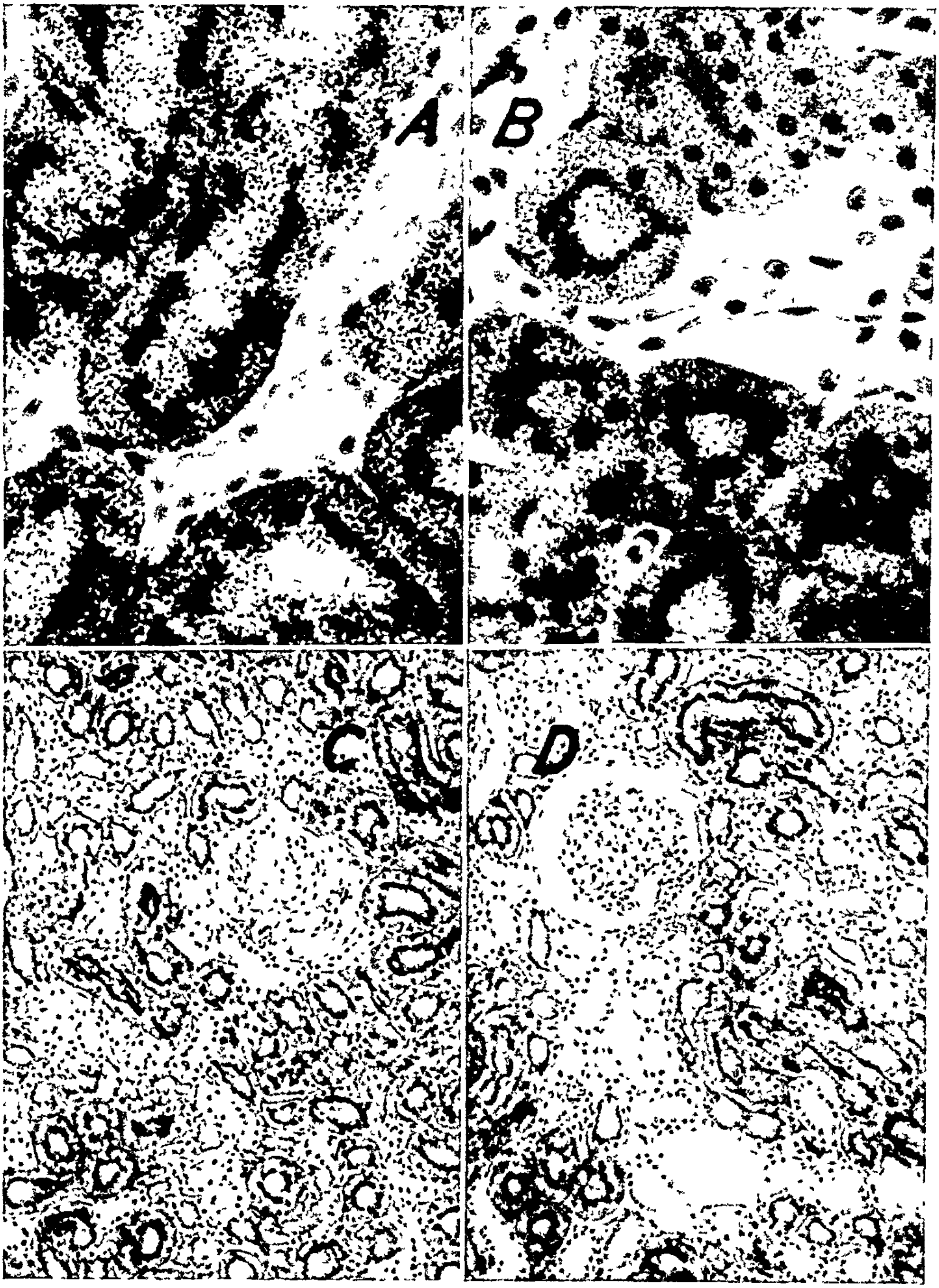

Fig. 1. Photomicrographs of kidney sections showing localization of "alkaline" phosphatase. A. Control rat, ×460; B. Acutely phlorizinized rat (marked glycuresis), ×460; C. Dog before injection of phlorizin, ×110; D. Same kidney 10 minutes after injection (marked glycuresis), ×110. Enzymic activity, indicated by granules in proximal convoluted tubules, is unaffected by phlorizin. Glomeruli, distal convoluted tubules and collecting ducts show no granules.

inhibition of the enzyme. When sections of normal rat kidneys were incubated in the presence of added phlorizin in concentrations up to M/100, no reduction in enzyme activity resulted.

DISCUSSION. There is no evidence that phlorizin glycuresis is due to inhibition of the "alkaline" phosphatase present in the proximal convoluted tubules. There is considerable recent support, on the other hand, for Lundsgaard's main thesis (5, 6, 14) that phosphorylation of glucose occurs in the course of tubular reabsorption; not through reverse catalysis of a phosphatase, however (15, 16), but by the action of a specific enzyme system, kidney phosphorylase (15, 17, 18, 19). The evidence for existence of such an enzyme in the kidney and the present concept of its action was reviewed recently by Cori (20) and by Kalckar (21). It would appear that kidney phosphorylase constitutes part of the specific cellular mechanisms postulated to explain the rapid transport of glucose from the lumen of the proximal convoluted tubules to the blood-stream. In this scheme, the rôle of the "alkaline" phosphatase of the proximal convoluted tubules is to dephosphorylate hexose-phosphoric esters. Our results suggest that it is not this latter step which is blocked by phlorizin. This is in accord with the work of Kalckar (15, 17) which indicates that phlorizin inhibits glucose reabsorption by blocking some phase of the action of kidney phosphorylase upon glucose.

SUMMARY

No significant inhibition of the "alkaline" phosphatase activity of the proximal convoluted tubules was found by histochemical or chemical methods in acutely and chronically phlorizinized rats and in dogs. Phlorizin glycuresis apparently is not due to blocking of dephosphorylation by "alkaline" phosphatase in the kidney tubules.

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THE EFFECTS OF SOME DRUGS ON THE CROSSED PHRENIC PHENOMENON

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Langendorff (1887) and Girard (1890) observed contractions of one-half of the diaphragm in rabbits and dogs after an ipsilateral semisection of the spinal cord above C_3 and severance of the contralateral phrenic nerve. Schiff (1894) and Porter (1895) showed that the crossing of the respiratory impulses occurred only at the level of the phrenic nuclei. Rosenblueth and Ortiz (1936) found that the interruption of afferent nerve impulses was not responsible for the phenomenon. The phenomenon occurred in the absence of asphyxia. Reversible transient crossed contractions were obtained by reversible blocks of the phrenic nerve with ether or direct currents. Crossing did not occur until all the motor fibers of the phrenic nerve were cut. Rosenblueth, Klopp and Simeone (1938) proved that the phenomenon was independent of the respiratory center by stimulating electrically the respiratory tract above C_3. It was their conclusion that crossed contractions occur directly as a result of blocking motor fibers, that the central changes are mediated by some process not requiring the conduction of nerve impulses, and that once the crossed path has been opened subsequent crossings are more readily obtained.

The present study was undertaken to determine the effect of some drugs on the crossed phrenic phenomenon.

METHOD. Rabbits and cats were employed. The experiments were performed under dial anesthesia (Ciba, 0.6 to 0.7 cc. per kgm., intraperitoneally). In all the animals the cord was semisected at C_2, producing a respiratory hemiplegia. The diaphragmatic contractions were recorded as described by Rosenblueth, Klopp and Simeone, by using Head's slips, which attach to the xyphoid cartilage. In the records obtained by this procedure, diaphragmatic contraction is denoted by upward excursions of the tracing. All drugs were given intravenously into the jugular or femoral veins. Atropine (1 mgm. per kgm.) was given before the injection of prostigmin or eserine. Acetylcholine solutions were prepared fresh from the crystalline hydrochloride. Stimulation of the vagus nerves was ac-

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complished with alternating shocks from a Harvard induction coil with 5 volts in the primary circuit, by means of shielded silver electrodes. Asphyxia was produced by attaching to the tracheal cannula a rubber balloon containing expired air.

Results. A. *Prostigmin.* Of the 2 cats and 9 rabbits on which observations were made, following the injection of prostigmin, permanent crossing was obtained in 1 cat and in 1 rabbit, reversible crossing was

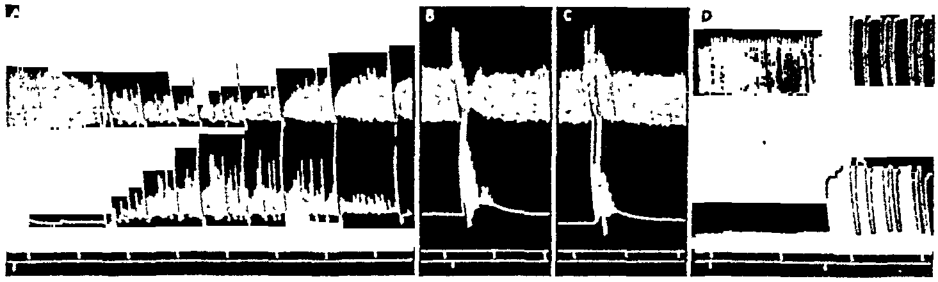

Fig. 1. Cat; dial; atropine (1 mgm. per kgm.). Left spinal semisection at C_2. Time signal: 30 sec. In this and the following figures the upper record corresponds to the right and the lower record to the left Head's diaphragmatic slip. The lower signals indicate the following procedures: A, prostigmin (0.5 mgm.); B, acetylcholine (100 γ)—injections of acetylcholine before prostigmin had not produced any crossed contractions; C, acetylcholine (100 γ) after denervation of the carotid sinuses; D, section of the vagi, first the left, then the right.

Fig. 2. Rabbit; dial; atropine (1 mgm. per kgm.); eserine (2 mgm. per kgm.). Left spinal semisection at C_2.

A, acetylcholine (200 γ); B, acetylcholine (300 γ) after denervation of the carotid sinuses; C, eserine (0.6 mgm. per kgm.).

noted in 1 cat (fig. 1A) and 1 rabbit, and no crossing was seen in 6 rabbits. After the injection of eserine (1.8 mgm.) reversible crossing was noted in 1 rabbit (fig. 2C). In the 2 animals that developed reversible crossing with prostigmin temporary crossed contractions had previously been elicited by asphyxia. Similarly, in the rabbit in which eserine caused crossed contractions previous crossings had been induced by acetylcholine and by block of the vagi.

B. *Acetylcholine.* Observations were made on 1 cat and 8 rabbits. In none of these animals did acetylcholine produce crossing when injected before administration of prostigmin or eserine. After injection of these drugs, however, acetylcholine (50 to 200 γ) caused transient crossed contractions in the 1 cat and in 7 of the 8 rabbits. In the cat and in 2 of the rabbits reversible crossing with acetylcholine after prostigmin (or eserine) occurred before and after denervation of the carotid sinuses (fig. 1B, C and fig. 2A, B). The changes in rate and amplitude of the respirations at the time of crossing with acetylcholine were inconsistent (figs. 1 and 2).

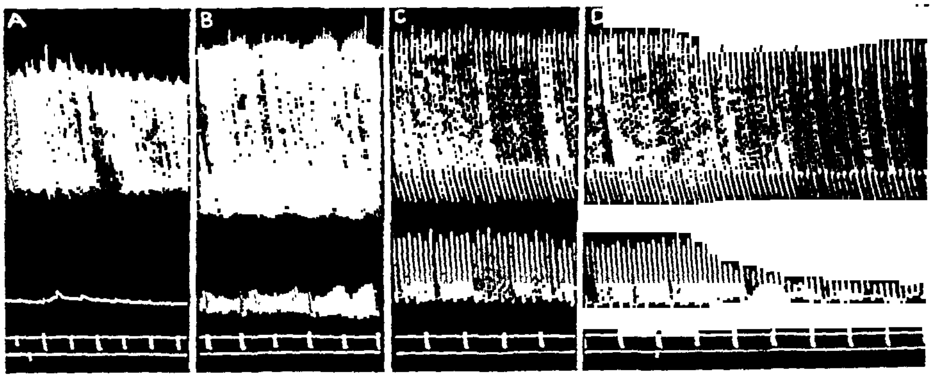

Fig. 3. Rabbit; dial. Left spinal semisection at C_2.
A, strychnine (0.2 mgm.); B, 7, and C, 90 minutes later; D, nembutal (6 mgm.).

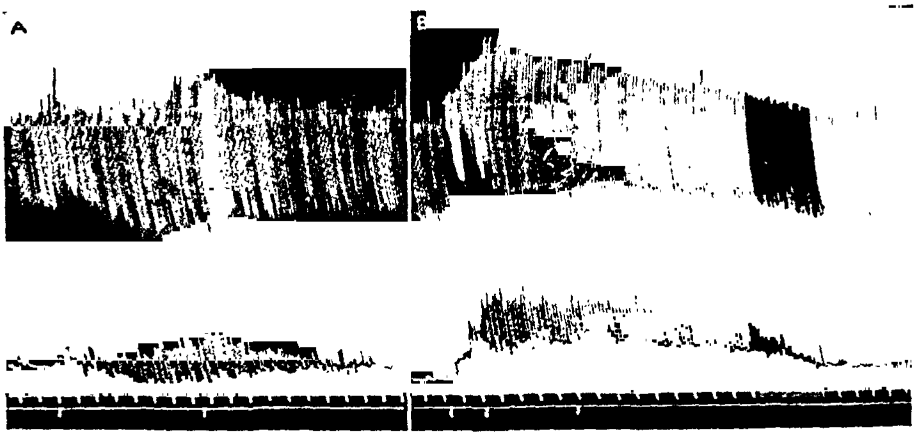

Fig. 4. Rabbit; dial; atropine (1 mgm. per kgm.); prostigmin (0.7 mgm. per kgm.). Left spinal semisection at C_2.
A, asphyxia; B, ether block of both vagi.

C. *Strychnine.* Observations were made on 4 rabbits. In 2 a permanent crossing took place after injection of 0.2 to 0.5 mgm. per kgm.; injection of nembutal (6 mgm.) to one of these rabbits depressed only the crossed contractions (fig. 3). In another animal crossed effects did not occur until 6 mgm. of strychnine had been given. The fourth rabbit did not exhibit the crossed phenomenon with convulsant doses of strychnine;

crossed contractions appeared later after injections of prostigmin and acetylcholine.

D. *Section of the vagi.* Rosenblueth and Ortiz (*loc. cit.*) reported that in cats and rabbits crossing was not observed after section of the vago-sympathetic nerves (13 animals). In the present observations, made on 2 cats and 6 rabbits, permanent crossing was noted in 1 cat after this section. A similar permanent crossing was produced by vagal section in 1 cat and 4 rabbits which had received prostigmin previously. In 2 rabbits no crossing occurred on section of the vagi, but later permanent crossing was seen after section of the contralateral phrenic nerve. Two of the prostigminized rabbits in which there was permanent crossing when the vagi were cut, had shown reversible crossed contractions on block of these nerves by ether and by induction shocks (fig. 4B).

E. *Asphyxia.* Observations were made on 2 cats and 8 rabbits. Crossing with asphyxia was seen in the 2 cats and 6 of the rabbits (fig. 4A). In one of the remaining rabbits no crossing took place with asphyxia, with strychnine, section of the vagi, prostigmin, or acetylcholine, and crossing only appeared after section of the phrenic nerve. The other rabbit showed crossed effects after a large dose of strychnine.

Discussion. Rosenblueth and Ortiz (1936) showed that there are species differences with regard to the crossed phrenic phenomenon. They observed the phenomenon in dogs, cats, rabbits and woodchucks, but failed to find it in monkeys or guinea pigs. Section of the vago-sympathetic nerves produced crossed contractions in dogs (7 out of 8 animals). This procedure was at no time effective in cats or rabbits (13 animals). In our experience permanent crossing appeared in 1 cat on section of the vagi after a reversible crossing with asphyxia. Another cat and 4 rabbits, in which reversible crossed contractions had been produced by prostigmin and by acetylcholine protected by prostigmin, showed permanent crossing on section of the vagi. The only rabbit that developed permanent crossing after prostigmin had had the vagi cut previously. This indicates that after prostigmin cats and rabbits behave similarly to dogs on section of the vagi.

Crossing was observed after prostigmin, and after acetylcholine when protected by prostigmin, in several of the animals studied. Carotid sinus stimulation was ruled out by denervation of the carotid sinuses in 3 cases. Adrenal stimulation was shown to be of no importance by the failure of adrenaline to produce crossing. Strychnine produced crossing in 2 out of 4 animals. In 1 rabbit crossing did not occur with any of the drugs and section of the vagi, but crossed contractions followed section of the phrenic nerve. It appears, therefore, that section of the active phrenic is the most effective means of producing crossed contractions.

The crossings during asphyxia or after vagal section could be the result

of an increase (temporal or spatial) of the output from the respiratory center. During the crossings with prostigmin, acetylcholine and strychnine there was no evidence in most of the records of such an increased respiratory output. In these cases, therefore, the action was exerted at the neuronal mechanisms in the spinal cord concerned in the crossing. It is possible that these drugs may enhance transmission in the central synapses involved.

SUMMARY

In cats and rabbits under dial anesthesia, with spinal semisections at C_2 and consequent ipsilateral respiratory hemiplegia, crossed respiratory contractions of the paralyzed hemidiaphragm were produced or promoted by prostigmin or eserine (figs. 1 and 2), acetylcholine protected by prostigmin (figs. 1 and 2), strychnine (fig. 3), section of the vagi after prostigmin (figs. 1 and 4), and asphyxia (fig. 4).

The data emphasize that differences previously noted between rabbits, cats and dogs, with respect to the crossed phrenic phenomenon, are only quantitative.

The most efficient factor for producing crossed contractions is the section of the phrenic nerve (p. 105).

The authors wish to express their appreciation of the advice and encouragement given by Dr. A. Rosenblueth.

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THE SELECTIVE UPTAKE OF BROMINE BY THE THYROID GLAND WITH RADIOACTIVE BROMINE AS INDICATOR[1]

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The ability of the thyroid gland to concentrate iodine has been clearly established by the early observations of Baumann, Marine and others (1) and more recently by investigations with radioiodine (2–5). Moreover. it has been shown recently that a large percentage of *normal circulating iodine* is *constantly* being removed by the thyroid gland. This was observed in studies in which "tracer"[3] amounts of radioiodine were introduced. As much as 65 per cent of the administered labeled iodine was removed by the thyroid gland in 24 hours (4).

This selective activity of the gland is not restricted to iodine, for it has recently been shown that the next higher halogen, ekaiodine, is retained somewhat similarly (6). These observations raised the question whether the next lower halogen, bromine, would also be stored in thyroid tissue. Although several attempts have been made to compare the bromine content of thyroid with that of other tissue, many of the older observations suffered from inadequate analytical procedures employed for the determination of bromine. More recently, however, Baumann, Sprinson and Marine have shown that the hyperplastic thyroid contains more bromine than the blood, whereas the resting colloid-containing gland has no more bromine than the blood (7).

Experimental. Radiobromine was produced by the neutron bombardment of bromobenzene containing 5 per cent by volume of aniline according to the method of Lu and Sugden (8). The bromobenzene-aniline solution was shaken with water, and the aniline was removed from the water layer by extraction with ether. In this manner a water solution of radiobromine containing very little bromine was obtained; it was estimated that each

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[3] A "tracer" dose of iodine refers to a sample of radioiodine containing no carrier. Such a sample of radioiodine contains only insignificant amounts of iodine (4).

millicurie of activity was accompanied by an amount of bromine of the order of magnitude of 10^{-6} mgm. Since each animal received less than 50 microcuries of radioactivity, the total bromine administered was negligible when compared with the amount present in the animal. In one experiment KBr was added in an amount that made the dose received by each animal equal to 0.12 mgm. of Br.

The bromine isotope used in this study was Br^{82}, which has a half-life of 34 hours (9). It was deemed advisable not to use Br^{80} (4.4 hr. half-life) because it undergoes an isomeric transition to Br^{80} with an 18 minute half-life (9). Before the radioactivity of any tissue was determined, Br^{80} was allowed to decay to a negligible concentration.

The solutions of radiobromine were administered intraperitoneally to rats and guinea pigs. At intervals thereafter the animals were anesthetized with nembutal. Blood samples were taken by heart puncture and the other tissues rapidly excised. All tissues were placed in large test tubes and thoroughly mashed by means of a stirring rod with an $AgNO_3$ solution (1 cc. of 0.15 M $AgNO_3$ per gram of tissue). After digesting this mixture on the steam bath for 1 hour, 3 cc. of concentrated HNO_3 were added for each gram of tissue and the heating continued for 8 hours. After diluting the digests with several volumes of water, the silver halides were filtered and mounted for the determination of radioactivity in the manner previously described for iodine (4). In order to equalize the mass of the precipitates of silver halides, 1 cc. of 0.02 M KBr was added in each case as carrier before digestion of the tissue was begun. This procedure was tested for recovery of bromine by the addition of radiobromine to inert tissue. The radiobromine was quantitatively recovered in all cases.

RESULTS. *Distribution of 0.12 mgm. of injected labeled bromine in the tissues of normal rats.* The content of radiobromine in tissues was measured at 2 intervals after the intraperitoneal injection of the sample of labeled bromine. The highest concentrations were found at both time-intervals in the *thyroid gland.* Thus at 3 hours after its introduction, 2 per cent of the administered labeled bromine was found in each gram of thyroid tissue, whereas 3 hours later 1.7 per cent was still present in each gram of this gland. Smaller amounts were found deposited in the liver, kidney, brain, salivary glands, pituitary and adrenals. Liver and kidney retained about equal amounts at the 2 time-intervals, namely, 0.7 to 0.8 per cent. Somewhat similar concentrations were found in the salivary glands and the pituitary. The lowest concentrations appeared in the brain and adrenal glands. Average values of 0.29 and 0.25 per cent were observed in the former, whereas each gram of adrenal tissue contained 0.37 and 0.31 per cent at 3 and 6 hours respectively. Whole blood contained higher amounts of the labeled bromine than all other tissues except thy-

roid. Thus at 3 and 6 hours after the administration of the labeled bromine 1.5 and 1.3 per cent of it was found in each gram of whole blood.

TABLE 1

The distribution of 0.12 mgm. of labeled bromine in the normal rat

(Each rat received intraperitoneally 1.5 cc. of a solution containing labeled bromine as KBr)

3 HOURS AFTER INJECTION				6 HOURS AFTER INJECTION			
Rat number*	Tissue	Br activity per gram†	Average	Rat number*	Tissue	Br activity per gram†	Average
8	Liver	0.95		11	Liver	0.62	
9	Liver	0.75		12	Liver	0.70	
10	Liver	0.63	0 78	13	Liver	0.71	
				14	Liver	0.69	0.68
8	Kidney	0 91		11	Kidney	0.78	
9	Kidney	0.89		12	Kidney	0.95	
10	Kidney	0.81	0.87	13	Kidney	0.71	
				14	Kidney	0.69	0.78
8	Brain	0.27		11	Brain	0.24	
9	Brain	0.28		12	Brain	0.28	
10	Brain	0.31	0 29	13	Brain	0.26	
				14	Brain	0.23	0.25
8	Whole blood	1.43		11	Whole blood	1.38	
9	Whole blood	1.53		12	Whole blood	1.11	
10	Whole blood	1.48	1 48	13	Whole blood	1.37	
				14	Whole blood	1.51	1.34
7, 8, 9, 10	Salivary glands‡		0.73	11, 12, 13, 14	Salivary glands		0.58
7, 8, 9, 10	Pituitary‡		0.65	11, 12, 13, 14	Pituitary		0.57
7, 8, 9, 10	Adrenals‡		0.37	11, 12, 13, 14	Adrenals		0.31
7, 8, 9, 10	Thyroid‡		2.02	11, 12, 13, 14	Thyroid		1.68

* Male rats weighing 200 grams were used.
† Refers to the per cent of the administered labeled bromine.
‡ Tissues pooled from 4 rats.

The distribution of a "tracer" dose of radiobromine in the tissues of normal guinea pigs and in guinea pigs treated with thyrotropic hormone. Twelve male guinea pigs weighing approximately 300 grams each were used in

this experiment. Six of these were treated with thyrotropic hormone[4] over a period of 10 days before the injection of radiobromine. Blood was taken by heart puncture after the animals had been anesthetized with nembutal. The blood was allowed to clot and serum removed by centrifugation. The gastrocnemius muscle was taken as the sample of muscle.

The highest concentrations of radiobromine were found in the thyroid gland of both normal and hormone-treated guinea pigs. This is particularly well shown at the 24-hour interval. The highest concentrations of radiobromine per gram of liver, muscle and adrenal gland did not exceed 0.32 per cent of the administered labeled bromine at the 2-hour interval,

TABLE 2

The distribution of a "tracer" dose of radiobromine in the tissues of normal guinea pigs and in guinea pigs treated with thyrotropic hormone†

TIME INTERVAL	ANIMAL NUMBER		THYROID WEIGHT		BROMINE ACTIVITY*													
					Thyroid				Adrenal		Liver		Muscle		Serum	
					Normal		Treated									
	Normal	Treated	Normal	Treated	Per gram	Whole organ	Per gram	Whole organ	Normal, per gram	Treated, per gram	Normal, per gram	Treated, per gram	Normal, per gram	Treated, per gram	Normal, per gram	Treated, per gram
hours			gram	gram												
2	1	7	0.045	0.152	0.68	0.031	0.880	0.134	0.234	0.178	0.112	0.167	0.114	0.111	0.469	0.561
2	2	8	0.055	0.108	0.52	0.029	0.816	0.0883	0.210	0.192	0.120	0.139	0.125	0.101	0.522	0.552
2	3	9	0.039	0.086	0.58	0.023	0.73	0.0629	0.191	0.321	0.120	0.170	0.104	0.141	0.506	0.704
24	4	10	0.040	0.091	0.52	0.021	0.614	0.056	0.148	0.141	0.102	0.0744	0.0706	0.0566	0.346	0.335
24	5	11	0.047	0.090	0.51	0.024	0.696	0.063	0.143	0.168	0.0855	0.0981	0.0742	0.0735	0.322	0.357
24	6	12	0.046	0.115	0.39	0.018	0.641	0.0737	0.138	0.139	0.0887	0.0844	0.0785	0.0670	0.362	0.358

* Refers to per cent of the administered labeled bromine.
† Each animal received subcutaneously 8 mgm. of thyrotropic preparation over a period of 10 days.

whereas the values per gram of thyroid tissues ranged from 0.52 to 0.88 per cent. At 24 hours the content of labeled bromine in liver, muscle and adrenal gland did not exceed 0.17 per cent per gram of tissue; at this time-interval thyroid contained from 0.40 to 0.70 per cent per gram.

No significant change in the uptake of labeled bromine by liver, muscle and adrenal gland resulted from treatments with thyrotropic hormone. Despite the fact that the thyroid gland showed a greater avidity for labeled bromine than the other tissues examined, hypertrophied glands produced

[4] We are indebted to Dr. Q. Bartz of Parke, Davis and Company, Detroit, for the thyrotropic preparation used in this investigation. This fraction assayed 4 guinea pig units per milligram. The unit is defined as the total dose in milligrams injected subcutaneously once daily for 4 days into 180 to 200 gram guinea pigs that produces on the fifth day minimal but definite hyperplasia of the thyroid in all of 6 animals.

by treatments with thyrotropic hormone failed to show striking increases in their uptake of bromine when compared with normal glands. Thus at the 24-hour interval the hypertrophied glands contained an average of 0.65 per cent of the administered labeled bromine per gram, as compared with 0.47 for normal glands. At the earlier interval the average values for normal and hypertrophied gland were respectively 0.59 and 0.81 per cent per gram of tissue.

COMMENT. It is generally accepted that administered chloride distributes itself principally, if not entirely, throughout the extracellular phase and can be used to determine the extent of these phases in a tissue.

TABLE 3

Calculation of extracellular phase based on distribution of labeled bromine

CONDITION	TIME	ANIMAL NUMBER	THYROID F	ADRENAL F	LIVER F	MUSCLE F
	hours					
Normal	2	1	123	43.8	20.2	20.6
	2	2	87.8	35.5	20.3	21.1
	2	3	101	33.2	20.9	18.1
Treated	2	7	138	28.0	26.2	17.5
	2	8	131	30.7	22.2	16.2
	2	9	91.5	40.2	21.3	17.7
Normal	24	4	132	37.7	26.0	18.0
	24	5	140	39.2	23.4	20.3
	24	6	95	33.6	21.6	19.1
Treated	24	10	162	37.2	19.6	14.9
	24	11	159	38.4	22.4	16.8
	24	12	158	34.2	20.8	16.5

Wallace and Brodie (10) compared $\frac{\text{tissue Br}}{\text{serum Br}}$ with $\frac{\text{tissue Cl}}{\text{serum Cl}}$ and concluded that the distribution of both halides is similar in all tissues examined except the brain. In the case of the brain, however, equilibrium is established with the halides in cerebrospinal fluid rather than with those in serum. It was concluded from these measurements that a selective retention of bromine by tissue cells is unlikely. Similar results were obtained by Weir and Hastings (11).

In table 3 are shown the values for the extracellular phase of tissues based on the distribution of radiobromine. The calculations were made according to Hastings and Eichelberger (12), the assumption being made for the present purpose that radiobromide distributes itself freely between

serum and the extracellular spaces only. The following equation was used in determining the extracellular phase of tissues:

$$F = \frac{Br_T \times 0.95 \times 0.92 \times 100}{Br_S \times 0.99}$$

where

F = Extracellular phase as grams per 100 grams of wet tissue,

Br_T = per cent of the administered labeled bromine recovered per gram of wet tissue,

Br_S = per cent of the administered labeled bromine recovered per gram of serum.

The figures 0.99 and 0.92 are values accepted for the fractions of water in the extracellular phase and serum respectively; 0.95 is the value for the Gibbs-Donnan ratio.

The average values for the extracellular phase of muscle, liver and adrenals obtained by this type of calculation are respectively 18.1, 22.1 and 36.0 grams per 100 grams of tissue. The results for muscle and liver agree satisfactorily with those of Weir and Hastings (11) and Wallace and Brodie (10). No values for the adrenals are available for comparison.

By this calculation, however, most of the values for extracellular phase, F, of thyroid tissue turn out to be greater than 100, i.e., greater than the entire mass of tissue. It is therefore apparent that the assumption upon which the F values for thyroid were determined is invalid. It may now be concluded that no simple relation exists between serum and thyroid bromine similar to that observed between serum and the other tissues examined.

SUMMARY

1. The uptake of bromine by various tissues of the rat and guinea pig was investigated with radioactive bromine as indicator.

2. The normal thyroid gland as well as the hypertrophied gland produced by injections of thyrotropic hormone showed a much greater uptake of labeled bromine than any of the other tissues examined. The content of radiobromine in the thyroid was too high to be explained by simple diffusion from serum.

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DEMONSTRATION OF VITAMIN A IN THE RETINA BY FLUORESCENCE MICROSCOPY

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Jancso and Jancso (1) described in a preliminary report the presence of green fading fluorescing droplets in the pigment coat of the light adapted eye of the albino rat, and the absence of such fluorescence from the dark-adapted eye. In accordance with the fluorescent microscopic study of Querner (2), they ascribed this fluorescence to vitamin A. Jancso and Jancso in correlating their findings with those of Wald (3) spoke of the reversible formation of vitamin A from visual purple.

The histological distribution of vitamin A in tissues of the albino rat has recently been more extensively studied (4, 5), and evidence was provided for the specificity of the green fading fluorescence in fixed tissue sections (for vitamin A); a short description of the retina of the light adapted eye was offered. In this paper we shall provide confirmation of the preliminary report of Jancso and Jancso, which, to our knowledge, has not yet been done.

METHODS AND MATERIALS. Under the fluorescence microscope a green fading fluorescence of lipoids is characteristic for vitamin A. The tissue is prepared by short formalin fixation. Frozen sections mounted in water are examined shortly after cutting.

The light is supplied by a mercury vapor bulb. The visible light is filtered out by a Corning Glass Filter no. 584, and a glass cell containing copper sulfate solution. An eyepiece filter cuts out the ultra-violet light after it has passed through the tissue.

Methylene blue staining does not significantly interfere with the fluorescence of vitamin A. Thus a methylene blue stained section can be examined both in visible and in ultra-violet light. This assists in the localization of the vitamin A fluorescence. A detailed description is given in a previous paper (4).

Light adapted eyes were prepared *in vivo* by placing the rat under a bright light for at least an hour. Dark adapted eyes were prepared *in vivo*

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by placing the rat in a dark room for at least two hours. Partially light adapted eyes were prepared by putting dark adapted eyes in light for a few minutes. The eyes of rats taken directly from the laboratory are referred to as laboratory adapted eyes.

The diets of the rats examined varied in their vitamin A content so that the rats were either completely deficient, partially deficient, normal or hypervitaminotic. Since 95 per cent of the vitamin A store is in the liver (6, 7), the nutritional state of the animal was evaluated by the vitamin A content of the liver as determined histologically and chemically.

One eye of each animal was examined histologically and the other macroscopically for visual purple content (colorimetric scale of Fredericia and Holm (8)).

RESULTS. On gross inspection the retina of the *laboratory adapted eye* was usually brick colored. Microscopically the vitamin A fluorescence was seen in the pigment coat in small droplets. The droplets were arranged around the periphery of the pigment epithelial cells and connected by green fluorescing filaments. The vitamin A fluorescence faded within a few seconds upon irradiation. Thus the hexagon shaped cells were outlined until the vitamin A fluorescence had faded to change all the pigment coat to the uniform non-fading green of the cytoplasm. Only the large centrally located nuclei imparted no fluorescence.

In the examined frozen sections the retina was usually separated from the pigment epithelium except at the papilla of the optic nerve and at the ora serrata. The outer segments of the rod and cone layer were usually torn and remained within the pigment coat when the retina and pigment coat were separated.

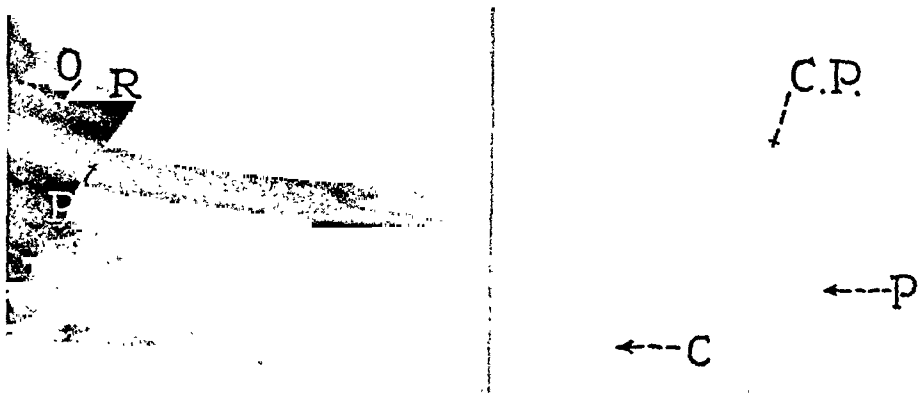
The rod and cone layer of the retina contained only minute traces of vitamin A. In addition, a green non-fading fluorescence was seen in the rod and cone layer and in the outer molecular layer of the retina. The nuclear layers were free of fluorescence. In the ciliary processes vitamin A fluorescence was seen in the interstitium: in the capillary endothelium and in the fixed connective tissue cells. The posterior portion of the ciliary body was rich in vitamin A fluorescence (fig. 1, A).

On gross inspection the retina of the *dark adapted eye* was deep red in color. Microscopically the vitamin A fluorescence of the pigment coat and of the rod and cone layer was either absent or only seen in traces. The green non-fading fluorescence was changed to a definite rust-brown color in the pigment coat, in the rod and cone layer, and in the outer molecular layer. The absence of droplets with vitamin A fluorescence from the pigment coat gave it a uniform appearance on contrast to the hexagonally outlined cells of the light adapted pigment coat. The vitamin A fluorescence of the ciliary body did not vary from that of the light adapted eye (fig. 1, B).

On gross inspection the retina of the *partially light adapted eye* was either pink or light red in color. Microscopically it was similar to the dark adapted eye except for a few traces of vitamin A fluorescence in the rust-brown fluorescing pigment coat.

The nutritional status of the rats did not significantly influence the fluorescence microscopic picture of the retina and the pigment coat. In vitamin A deficiency the vitamin A fluorescence might have been reduced but the fluorescence was never completely absent from the light adapted eyes; not even in the ulcerated eyes in extreme vitamin A deficiency (table 1).

In hypervitaminosis there was no apparent increase in the vitamin A fluorescence of the retina and pigment coat during light adaptation and the vitamin A fluorescence was absent during dark adaptation.



A B

Fig. 1A. Section of posterior wall of a light adapted eye of an albino rat. Vitamin A fluorescence localized in: *P*, the pigment coat, and *R*, the rod and cone layer. Non-fading green fluorescence in the outer molecular layer, *O*.

Fig. 1B. Section of dark adapted eye. Vitamin A fluorescence in the interstitium of the ciliary processes, *C. P.* The pigment coat, *P*, shows the dim non-fading rust-brown fluorescence. The blue fluorescence of the cornea, *C*, (appearing white in the black and white photograph) should not be confused with the vitamin A fluorescence.

The vitamin A fluorescence of the ciliary processes did depend upon the vitamin A nutritional status. It was absent in vitamin A deficient and hypovitaminotic animals. Its amount was increased in hypervitaminosis.

The vitamin A fluorescence and the green non-fading fluorescence of the light adapted eye and the rust-brown fluorescence of the dark adapted eye were not changed by formalin fixation (for not more than 10 hrs.) or by *in vitro* treatment with hydrogen peroxide, sodium hydrosulfite (as reducing agent), or normal ammonium hydroxide. Alcohol and acetone removed the vitamin A fluorescence and the non-fading green and yellow-brown fluorescence of pigment coat and retina. Dilute hydrochloric acid and glycerine left the vitamin A fluorescence unchanged, but with the former

the non-fading green and rust-brown fluorescence was made even brighter while with the latter the non-fading green and rust-brown fluorescence was changed to a red.

The cornea, lens and the other tissues of the eye did not show vitamin A fluorescence except for the adjacent fat cells which did show it in accordance with the nutritional status.

DISCUSSION. There are two types of vitamin A distribution in the eye: 1. In the ciliary body the vitamin A fluorescence is seen in the interstitial elements such as the connective tissue cells and the capillary endothelium; the distribution is similar to that of the meninges, serous membranes, lungs, etc. (5), and the fluorescence reflects the nutritional status of the animal. 2. In the pigment coat and in the retina there is a specific vitamin A distribution, which is practically independent of the nutritional status. It is instead dependent upon the state of light adaptation as described by

TABLE 1

Vitamin A fluorescence of the pigment coat and rod and cone layer of the retina of 86 albino rats under varied nutritional conditions and under varied states of light adaptation

VITAMIN A FLUORESCENCE IN THE RETINA	VITAMIN A FLUORESCENCE OF THE LIVER							
	Absent		Traces		Normal		Hypervitaminotic	
	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
Laboratory adapted eyes	8		20		10		5	
Light adapted eyes	2				7		4	
Partially light adapted eyes	2				5			
Dark adapted eyes		8		2		9		4

Jancso and Jancso. Possibly there are reduced amounts in vitamin A deficiency; but complete absence of vitamin A has never been seen by us. Hemeralopia, then, is not the result of a complete absence of vitamin A. A definite reduction of vitamin A is not necessarily seen histologically. These findings therefore neither affirm nor contradict the adaptometric studies.

The vitamin A in the second location depends upon the functional state. According to Wald it is formed from the visual purple of the outer segments of the rods in light adaptation. There is an intermediate state during which the visual purple is changed to visual yellow in a reversible reaction. The visual yellow is broken down to colorless end-products, among which is vitamin A. The vitamin A is in part used again in the slow transformation back to visual purple.

The histological studies are in agreement with Wald's cycle in that the vitamin A fluorescence is seen in light adaptation in concentrations de-

pending upon the degree of light adaptation; in that it is absent in complete dark adaptation; in that by far the strongest fluorescence is seen in the pigment coat and not in the rod and cone layer. Grossly these eyes showed high amounts of visual purple in the retina in dark adaptation, lesser in partial adaptation and none in light adaptation. Whether or not the non-fading rust-brown fluorescence is due to visual purple or visual yellow cannot be determined.

The visual purple or visual yellow droplets demonstrated in visible light (9, 10, 11) were not seen in fluorescence microscopy. We saw visual purple macroscopically (in accordance with Holm, 12) and vitamin A histologically in severe vitamin A deficiency contrary to Tansley (13) and Johnson (14) who report its absence.

CONCLUSIONS AND SUMMARY

There are two types of vitamin A distribution in the eye as shown by fluorescence microscopy, namely: *a*, in the ciliary processes where it depends on the nutritional status and where it is independent of the state of light adaptation; and *b*, in the pigment coat and in the rod and cone layer of the retina where it is functional. Here the presence of vitamin A does not depend on the nutritional state. Possibly there is a reduction in amount in vitamin A deficiency. As described by Jancso and Jancso the presence of vitamin A depends on the state of light adaptation. It is absent in dark adaptation. These observations are in agreement with Wald's cycle describing the rôle of vitamin A in vision.

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WEIGHT CHANGES IN THE CORTEX AND THE MEDULLA OF THE ADRENAL GLAND OF THE DOG IN ACUTE VITAMIN-B_1 DEFICIENCY

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Numerous investigators have shown that there are many pathological conditions in which the adrenals hypertrophy while other endocrine glands atrophy. Outstanding among the conditions in which this enlargement is reported to occur are injections of hormones, avitaminoses, inanition and infection (1–6). Histological changes in both the cortex and medulla have been described, but there is no clear cut evidence indicating whether these changes consistently involve the cortex, the medulla or both. From the variety of changes which have been reported it seems probable that the part of the gland affected may depend upon either the nature or the severity of the pathological condition. Evidence for this is given by Deansley (6) who made a study, not only of the histological picture, but also of the volume changes in the cortex and medulla of the adrenals in mice when given injections of thyroxine, morphine or killed B. gaertner.

Although many workers have reported enlargement of the adrenals in vitamin-B_1 deficiency, data are lacking in regard to the extent of the involvement of each part of the gland. Sure (4) has reported a total enlargement of the adrenals in rats in vitamin-B_1 deficiency which was 50 per cent greater than that of the controls kept at the same plane of nutrition.

In this study an attempt has been made to determine weight changes in both parts of the gland in acute vitamin-B_1 deficiency in the dog so that the portion responsible for the increased weight may be known.

METHODS. Seven young mature dogs weighing from 5 to 14 kgm. were used. Each dog was wormed, immunized against distemper, and maintained on a normal diet for at least one week before the deficient ration was started. This ration, which was fed until acute symptoms of vitamin-B_1 deficiency appeared, consisted of:

	Per cent
Casein	19
Sucrose	60

	Per cent
Cottonseed oil	8
Salt mixture (186)*	4
Autoclaved yeast	8
Agar	1
Cod liver oil concentrate†	

* Manganese and copper added.
† Squibbs Adex Tablets—1 tablet per day.,

The ration was given ad libitum, the average daily consumption during the first week on the diet being 150 grams. After this time the food consumption became more variable. Five to six days before the appearance of acute symptoms the food intake averaged approximately 30 grams. Table 1 shows the initial and final body weights of each dog and the time required to produce severe symptoms of polyneuritis in each case (36–59 days). Prior to the onset of acute symptoms frequent attacks of vomiting occurred. Three to four days later the dogs became extremely spastic and presented the characteristic symptoms of acute B_1 deficiency. All dogs were allowed to live 24 hours after the appearance of these symptoms, then killed, and the adrenals removed immediately.

Determination of weights of the cortex and medulla. In order to save the cortex of the glands for physiological tests the following procedure was devised for determining the weights of the cortex and the medulla. Each pair of glands was dissected free of connective tissue and fat in a moist chamber and weighed in a weighing bottle. Each gland was cut in half longitudinally and the medulla, which stood out clearly from the cortex, was carefully teased out by blunt dissection. All of the adrenal glands removed from the vitamin-B_1 deficient dogs as well as from the controls were weighed in this manner (tables 1 and 2).

To establish the range of variation of these ratios in normal dogs, the adrenals from 96 dogs obtained from the pound were dissected and weighed. Each dog was classified as to approximate age (immature, mature, or old), sex, general health, and, in the case of females, its stage in the estrual cycle. All dogs were weighed immediately after being killed.

Baker (7) has shown that the weight of the adrenal glands varies with the age and the weight of the animal and that the ratio of medulla weight to cortex weight is approximately the same in mature males as in females in dioestrus. Hence, as a basis for comparison, only those dogs were chosen that were healthy, mature, in dioestrus, in the case of females, and within the same weight range as those in the series of B_1 deficient animals (table 2). All of the data were analyzed statistically for significance of difference, using a formula for small samples given by Tippett (8).

Results and discussion. A common method used to indicate the size of an organ is to express it as a per cent of the body weight. Com-

parison of the percentage organ weights for the vitamin-B_1 deficient animals with those for normal animals will indicate whether any change in size has occurred. As a loss of body weight usually accompanies vitamin-B_1 deficiency, the question arises whether the initial or the final body weight should be used in making these computations. Even though there is no actual change in size of an organ, if a marked loss of body weight occurred during the experimental period, the values obtained by expressing the organ weight as a per cent of the *final* body weight will be larger than those for normal animals.

As McCarrison (1) and Jackson (5) have shown that the loss of body weight in the various avitaminoses is due not only to a loss of body fat but also to atrophic changes in the organs and tissues, it should be expected that a loss of weight of the endocrine glands would also occur. Hence, the

TABLE 1

Showing weights of adrenal glands in relation to body weight in vitamin-B_1 deficient dogs

DOG NUMBER	SEX	DEPLETION TIME	INITIAL BODY WT.	FINAL BODY WT.	GLAND WT.	CORTEX WT.	MED. WT.	GLAND WT. PER CENT INITIAL BODY WT.	GLAND WT. PER CENT FINAL BODY WT.	CORTEX WT. PER CENT INITIAL BODY WT.	CORTEX WT. PER CENT FINAL BODY WT.	MED. WT. PER CENT INITIAL BODY WT.	CORTEX WT. / MED. WT.
		days	*kgm.*	*kgm.*	*gm.*	*gm.*	*gm.*						
1	F	36	7.26	8.35	0.922	0.856	0.066	0.013	0.011	0.012	0.010	0.0010	13.0
2	M	37	7.09	6.40	0.901	0.826	0.081	0.013	0.014	0.012	0.013	0.0011	10.2
3	M	39	5.38	5.87	0.941	0.895	0.046	0.017	0.016	0.017	0.015	0.0010	10.8
4	M	48	7.79	6.46	1.367	1.256	0.111	0.017	0.021	0.016	0.019	0.0014	11.3
5	M	49	8.87	6.89	0.991	0.902	0.089	0.012	0.014	0.010	0.013	0.0010	10.1
6	M	59	10.4	7.83	1.207	1.082	0.125	0.012	0.015	0.011	0.014	0.0012	8.6
7	M	55	13.9	8.49	1.649	1.521	0.128	0.012	0.019	0.011	0.018	0.0010	11.8

maintenance of the weight of a gland under conditions in which other organs are losing weight can be considered a *relative* hypertrophy. On the other hand, the hypertrophy can be considered as *absolute* if the weight of the gland, when expressed as a per cent of the *initial* body weight, is greater than that for normal animals.

In order to take both of these factors into consideration computations were made both on the basis of the initial and of the final body weights (table 3). It can be seen that the increase in size of the gland is due to the cortex, which undergoes an absolute (3A) as well as a relative (3B) hypertrophy.

On the other hand, the medulla weights of the adrenals of the B_1 deficient dogs, when expressed as a per cent of the initial body weight are lower than those for the normal animals, showing a tendency for this part of the gland

to follow the same trend as most of the organs and tissues (table 3A). For this reason the atrophy would not be apparent if the medulla weight

TABLE 2

Showing variation in weight of adrenal glands in relation to body weight in normal mature dogs

DOG NUMBER	SEX	BODY WT.	GLAND WT.	CORTEX WT.	MED. WT.	GLAND WT. PER CENT BODY WT.	CORTEX WT. PER CENT BODY WT.	MED. WT. PER CENT BODY WT.	CORTEX WT. / MED. WT.
		kgm.	*gm.*	*gm.*	*gm.*				
1	M	7.27	0.962	0.815	0.147	0.013	0.011	0.0021	5.5
2	M	8.60	1.060	0.929	0.131	0.011	0.011	0.0013	7.1
3	M	9.30	1.053	0.878	0.175	0.011	0.009	0.0019	5.0
4	M	12.70	1.190	0.988	0.202	0.009	0.008	0.0016	4.0
5	M	7.97	1.70	1.320	0.387	0.021	0.016	0.0040	3.4
6	M	12.20	1.510	1.340	0.176	0.012	0.015	0.0014	7.6
7	M	12.0	1.320	1.116	0.204	0.011	0.009	0.0017	5.5
8	M	8.80	0.903	0.689	0.214	0.010	0.008	0.0023	3.4
9	M	8.30	0.993	0.838	0.055	0.012	0.010	0.0066	15.1
10	M	7.27	1.146	0.991	0.155	0.016	0.014	0.0021	6.4
11	M	11.13	0.909	0.767	0.142	0.008	0.006	0.0014	5.4
12	M	7.38	0.789	0.722	0.067	0.010	0.010	0.0009	10.7
13	M	7.72	1.045	0.941	0.104	0.013	0.012	0.0013	9.3
14	M	11.37	0.954	0.817	0.137	0.008	0.007	0.0012	5.9
15	M	11.30	0.792	0.705	0.087	0.007	0.006	0.0008	8.1
16	M	9.10	0.715	0.635	0.080	0.008	0.009	0.0009	7.9
17	M	12.05	1.110	0.965	0.145	0.009	0.008	0.0012	6.6
18	M	12.50	1.220	1.030	0.190	0.010	0.008	0.0015	5.4
19	M	13.0	1.362	1.217	0.145	0.010	0.009	0.0011	8.4
20	M	12.50	1.169	0.968	0.201	0.009	0.008	0.0016	4.8
21	F	6.20	0.659	0.571	0.088	0.010	0.009	0.0014	6.5
22	F	9.70	1.219	1.076	0.143	0.013	0.011	0.0015	7.5
23	F	8.60	0.935	0.848	0.087	0.011	0.010	0.0010	9.8
24	F	8.80	1.30	1.113	0.187	0.015	0.013	0.0021	6.0
25	F	5.90	0.705	0.531	0.174	0.012	0.009	0.0030	3.3
26	F	7.72	0.950	0.786	0.164	0.012	0.010	0.0021	4.8
27	F	7.50	0.932	0.786	0.146	0.012	0.010	0.0020	5.4
28	F	6.58	1.044	0.879	0.165	0.016	0.013	0.0025	5.3
29	F	9.09	1.382	1.197	0.185	0.015	0.013	0.0020	6.5
30	F	6.80	1.180	0.982	0.199	0.017	0.014	0.0030	4.9
31	F	10.11	1.029	0.915	0.114	0.010	0.009	0.0011	8.0
32	F	8.40	1.115	0.930	0.184	0.013	0.011	0.0022	5.0
33	F	11.37	1.012	0.878	0.134	0.009	0.008	0.0012	6.6

were expressed as a per cent of the *final* body weight, as the loss in total body weight would mask the loss in weight of the medulla.

An increase is also noted in the ratio of the weights of cortex to medulla

(table 3B). Baker (7) from a comprehensive study of the weights of the adrenals of 1250 dogs reported that the weights of cortex to medulla were approximately 5 to 1 in both sexes. The results for normal dogs obtained in this study showed average ratios of 6 to 1. Considering the comparatively crude method employed, that the cortex might be saved for further work, this ratio corresponds well with Baker's. In the B_1 deficient dogs the average ratio for weight of cortex to medulla was 10.8 to 1 which is significantly higher than that obtained for the normal dogs. It can be seen that the increase in the ratio could be due either to an increase in cortex weight or to a decrease in medulla weight. From the results shown in table 3B it seems probable that both factors are involved.

TABLE 3

Comparison of adrenal glands of normal and of vitamin-B_1 deficient dogs

A. Expressed as per cent initial body weight

	NUMBER OF DOGS	GLAND WEIGHT PER CENT	STAN. DEV.	t	p	CORTEX WEIGHT PER CENT	STAN. DEV.	t	p	MED. WEIGHT PER CENT	STAN. DEV.	t	p
B_1 def.	7	0.014	0.0024	2.41	0.04	0.0125	0.0024	2.42	0.04	0.0011	0.0002	2.4	0.04
Normal	33	0.011	0.0025			0.0101	0.0023			0.0017	0.0006		

B. Expressed as per cent final body weight

	NUMBER OF DOGS	GLAND WEIGHT PER CENT	STAN. DEV.	t	p	CORTEX WEIGHT PER CENT	STAN. DEV.	t	p	CORTEX / MEDULLA	STAN. DEV.	t	p
B_1 def.	7	0.016	0.0030	4.0	0.01	0.0145	0.0028	4.2	0.01	10.8	1.29	5.1	0.01
Normal	33	0.011	0.0025			0.0101	0.0023			6.0	2.36		

As the results presented above indicate that the cortex is responsible for the enlargement of the adrenal glands in vitamin-B_1 deficiency in the dog, the question arises whether this necessarily means that there is an increased secretory activity of this part of the gland. Some workers have reported histological changes in the cortex under various pathological conditions which they have interpreted as an indication of increased activity. But as yet there is no direct evidence to justify this assumption. In a paper to follow, an attempt has been made to correlate weight changes of the cortex in vitamin-B_1 deficiency with changes in activity as indicated by a biological test.

SUMMARY

1. The adrenal cortex undergoes both a relative and an absolute hypertrophy in acute vitamin-B_1 deficiency in the dog.

2. The adrenal medulla shows a tendency to atrophy.

3. The ratio of the weight of cortex to medulla in normal dogs was found to be 6 to 1 whereas in the vitamin-B_1 deficient dogs it was 10.8 to 1.

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CHANGES IN CONCENTRATION OF STEROID COMPOUNDS IN THE ADRENAL CORTEX OF THE DOG IN VITAMIN-B_1 DEFICIENCY AS INDICATED BY THE BITTERLING TEST

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One of the outstanding changes described in the adrenal cortex in vitamin-B_1 deficiency is the change in the distribution of lipoids. McCarrison (1) and Kellaway (2), on the basis of histological studies, reported a wider distribution of the lipoids in the cortex in avian beri beri. Findlay (3) found a similar picture in the rat. That this change might conceivably be related to functional activity seems probable, as the various compounds which have been isolated from the cortex and shown to maintain life in an adrenalectomized animal are steroid compounds and would therefore be contained in the lipoid fraction of the gland. Zwemer (4), who made a study of adrenal morphology in various animals under both normal and pathological conditions, relates the wider distribution of lipoids to increased activity of the gland. Hoerr (5), on the other hand, does not think there is any good morphological evidence for indicating the function of the adrenal lipoids. Whitehead (6, 7) has shown that species variation must be considered in interpreting changes in cortical lipoids, and that the distribution changes with sex and age. As it is debatable whether changes in morphology of the gland should be used to gauge its functional state a physiological approach to the problem seemed desirable.

In 1936 Barnes, Kantner and Klawans (8) reported that aside from the gonads the adrenal glands were the only tissue which would yield an extract causing a lengthening of the ovipositor of the Japanese bitterling. Other investigators have shown that this reaction can be produced at will by various androgens and estrogens, and also by pure corticosterone (9 to 13). These results suggest that the bitterling test, though not specific for any one steroid compound, can be used to indicate the presence of this particular group of compounds. As this test can be made roughly quantitative it might serve as a useful criterion of the functional state of the adrenal glands until the identity of the true cortical hormone (or hormones) has been established.

Methods. A. *Measurement of ovipositor lengths.* Kleiner, Weisman

and Mishkind (14) and DeWit (11, 15) determined the extent of elongation of the ovipositor of the bitterling by visual comparison with the length of the rays of the anal fin. Since the ovipositor may elongate to a distance two or three times the length of these rays this method is only suitable for detecting gross changes. In order to make the more accurate measurements necessary for quantitative study, an objective record of ovipositor lengths was obtained by photographing the ovipositor against a millimeter ruler at constant position and magnification (2×). This was done by holding the fish in a glass compartment against the side of a square glass container. The negatives were projected onto a sheet of paper (enlargement 100×), and the outline of the ovipositor traced. This outline was then marked off with a marking wheel and the ovipositor length recorded in terms of the number of spacings made by the wheel. By photographing the ovipositor of one fish ten times and measuring each negative by this method the standard deviation was 4.3 (based on an average of 131 spacings).

To eliminate the element of variability in initial ovipositor lengths all data are expressed as per cent lengthenings over the initial lengths; in each case the initial length was determined prior to placing the fish in the solution. During the experimental period the length was determined at approximately 12-hour intervals. By using a running-water bath the temperature was maintained between 17° and 19°C. throughout the time these experiments were carried out (Jan. to May, 1940).

B. *Preparation of lipoids from the adrenal cortex.* The glands used to test the bitterling response were obtained from the dogs of the previous investigation, on which measurements of the weight changes in acute vitamin-B_1 deficiency had been made. The cortex of the gland, which had been dissected away from the medulla, was thoroughly ground with ether at room temperature. Ten cubic centimeters of ether per gram of tissue was used and the grinding continued for ten minutes. This procedure was carried out three times to insure complete extraction of the lipoids. Aliquots representing known amounts of cortex were pipetted into liter beakers. After evaporating to dryness the extract was suspended in 100 cc. of water at 55°C. and the solution made up to 500 cc. with water from the aquarium. Two fish were placed in each beaker after the solutions had reached the temperature of the water in the aquarium.

C. *Standardization of the bitterling response.* In order to establish differences in the concentration of the active compounds in different glands it was necessary to work below the concentration yielding a maximal lengthening. Barnes et al. (8) reported that the extract from 0.75 to 1.0 gram of adrenal cortex per liter of water was necessary to produce lengthening of the ovipositor. In some preliminary experiments, performed during the fall months to check their results, it was found that a concentration of

less than a gram of cortex per liter of water failed to produce consistent lengthening in all of the fish treated. Consequently this was the lowest concentration which could be used at this time. In order to determine whether concentrations greater than 1:1000 would produce proportionately greater lengthening, solutions were prepared with the aliquots representing one, two and three grams of cortex, each suspended in 1000 cc. of water. Four fish were treated with each concentration. It can be seen from figure 1 that there is less difference between the 3:1000 and the 2:1000 concentrations than between the 2:1000 and the 1:1000 concentrations. Thus the 3:1000 is approaching the concentration that will produce a maximal response. With the 1:1000 concentration not only is the total lengthening less but also a regression of the ovipositors begins at the end of 48 hours. No regression is noted in the ovipositors of the fish treated with the two higher concentrations even at the end of 66 hours. Treatment was stopped after this period of time because previous experiments had shown that if the fish were left longer in solutions of high concentrations, many of them died. On the basis of these observations the 1:1000 concentration was considered sufficiently below the maximal concentration and was used in the experiments performed during the month of February, 1940.

As Kleiner et al. (9) reported a change in sensitivity of the fish with the approach of the breeding season (March to August), a second standardization of the bitterling response was made in March, 1940, before beginning the assay of adrenal cortical steroids from the second group of dogs. Concentrations of 1:1000 and 1:2000 were used, six fish being treated with each concentration. Since these lower concentrations were not injurious to the fish, treatment was continued for 90 hours in order to obtain a more complete picture of the regression of the ovipositors. The results given in figure 2 show that the 1:2000 concentration produced a lengthening of the ovipositors of approximately the same magnitude as the 1:1000 concentration had produced in the previous standardization. And again there were differences both in the per cent lengthening of the ovipositors and in the time at which regression occurred. Since the 1:1000 concentration produced considerably greater elongation in this month than in the preceding month it was thought advisable to use the lower concentration in subsequent experiments.

In assaying the cortical extract from the normal and the vitamin-B_1 deficient dogs the per cent lengthenings of similar groups of fish treated during the same time interval were compared. This seemed preferable to comparing responses of the same group of fish treated first with extract from normal dogs and then a month later with that from B_1 deficient animals for the following reasons: 1, no fish could be treated oftener than once a month, as previous experiments had shown less consistent responses when tests were run at more frequent intervals; 2, the death of any of the

fish would alter the character of the groups, and 3, the change in age and condition of the fish would cause changes in sensitivity to the extract.

RESULTS. *Group I.* In February, 1940, 11 fish were treated with the extracts of the adrenal cortex from three dogs in which acute symptoms of vitamin-B_1 deficiency had been produced (table 1). During the same month, ten fish were treated with extracts from the adrenals of normal dogs. In figure 3 the average per cent lengthenings of the groups of fish are plotted against time. Approximately a 43 per cent increase in ovipositor lengthening is noted in the fish treated with the extract from the adrenals of the B_1 deficient dogs over that obtained in the fish treated with the extract from the glands of normal dogs.

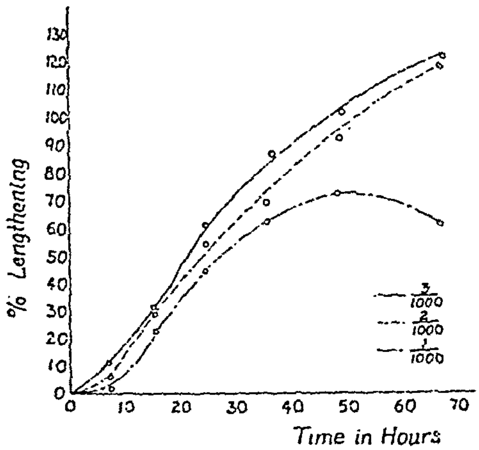

Fig. 1

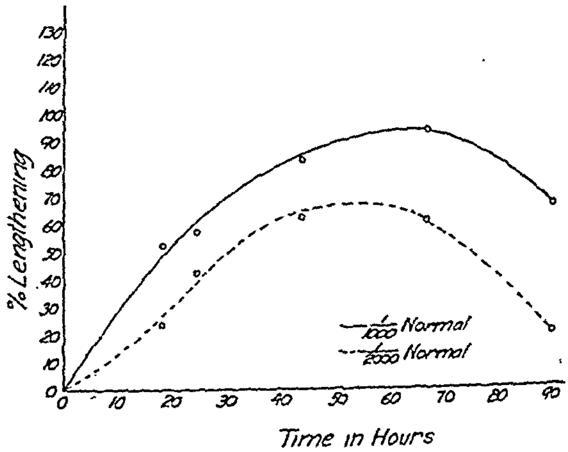

Fig. 2

Fig. 1. Bitterling response, February, 1940, to different concentrations of extract of adrenal cortex from normal dogs.

Fig. 2. Bitterling response, March, 1940, to different concentrations of extract of adrenal cortex from normal dogs.

Group II. In March, 1940, the responses of 15 fish treated with extracts from the adrenals of two dogs suffering from acute vitamin-B_1 deficiency were compared with those of 20 fish treated with extracts from the glands of normal dogs (fig. 4). Again, there is a much greater lengthening of the ovipositors of the fish treated with the extract from the adrenals of the B_1 deficient animals (about 50 per cent greater lengthening than in the controls).

Group III. Eighteen fish were treated with the extracts from the adrenals of two B_1 deficient dogs at the end of April, 1940. During the same time interval 17 fish were treated with the extracts from the glands of normal dogs. Results are shown in figure 5. The difference between the maximal per cent lengthening is not as great as in the two former groups (10 per cent). The smaller difference may have resulted from a further change in sensitivity of the fish due to approach of the breeding

season. This theory is borne out by the progressive increase in per cent lengthening of the ovipositors of the fish treated with the extract from the glands of normal dogs in successive months (table 1). If the sensitivity had increased, the concentration of 1:2000 of the extract from the adrenals of normal dogs was not sufficiently below that concentration necessary to evoke a maximal response and may even have been sufficient to produce maximal responses in some of the fish. However, the onset of regression of the ovipositors was later than in the controls, a factor which was shown by the standardization to vary with the concentration of extract used, and one which can be used to make reliable comparisons.

COMMENT. In analyzing the data statistically two factors have been considered: 1, the maximum per cent lengthening, and 2, the per cent

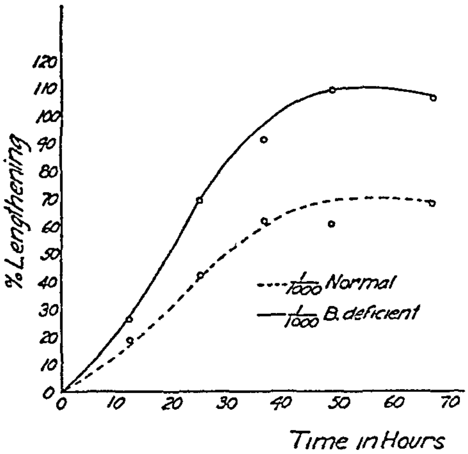

Fig. 3

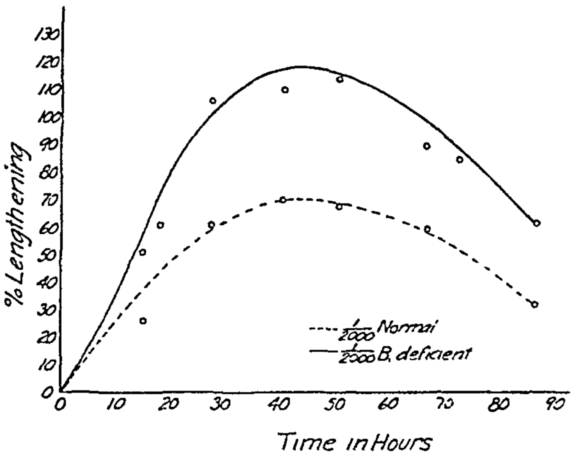

Fig. 4

Fig. 3. Bitterling response, group I, February, 1940, to 1:1000 concentration of adrenal cortical extracts.

Fig. 4. Bitterling response, group II, March, 1940, to 1:2000 concentration of adrenal cortical extracts.

lengthening at the end of 60 hours. Analysis of the results (Tippett, 16) based on the maximum per cent lengthening of the ovipositors shows that in groups I and II there are significant differences between the responses of the fish treated with the extract from the adrenals of the B_1 deficient dogs and those of the fish treated with the extract from the adrenals of normal dogs (table 1). In group III, which was run during April, the ten per cent difference over the normal in the maximum per cent lengthening of the ovipositors is not great enough to be statistically valid.

The second comparison, of the per cent lengthening of the ovipositors at the end of 60 hours, was chosen because regression of the ovipositors had begun in all of the fish by this time. In groups I and II this analysis gives almost identical results with those of the preceding method. By employing this method of analysis it is possible to validate the differences in the third group statistically (table 1). Thus a significant change in the

steroid compounds in the third group has been demonstrated in spite of the increased sensitivity of the fish. Due to a scarcity of fish it was impossible to carry out a third standardization at this time.

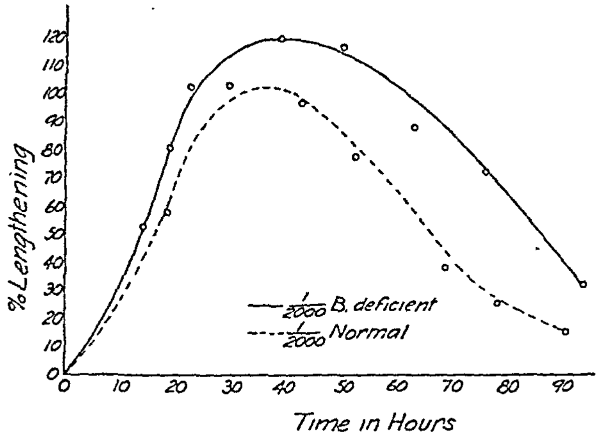

Fig. 5. Bitterling response, group III, April, 1940, to 1:2000 concentration of adrenal cortical extracts.

TABLE 1

Comparison of effects on bitterling response of extract of adrenals from normal and from vitamin-B_1 deficient dogs

GROUP	NUMBER OF DOGS	DEPLETION PERIOD	MONTH TREATED	CONCENTRATION OF SOLUTION	NUMBER OF FISH	MAXIMUM PER CENT LENGTH	STAN. DEV.	t	p	PER CENT LENGTH AT 60 HOURS	STAN. DEV.	t	p
		days											
I													
B_1 def.	3	37											
		38	Feb.	1:1000	11	115.0	19			105.0	26		
		40						4.0	0.01			3.6	0.01
Normal	2	0	Feb.	1:1000	10	79.0	19			69.0	15		
II													
B_1 def.	2	48											
		49	Mar.	1:2000	15	121.0	38			96.0	41		
								3.5	0.01			3.3	0.01
Normal	4	0	Mar.	1:2000	20	81.3	25			58.0	29		
III													
B_1 def.	2	55											
		59	Apr.	1:2000	18	126.0	27			89.0	38		
								1.0	0.3			2.68	0.02
Normal	3	0	Apr.	1:2000	17	116.0	34			57.0	29		

DISCUSSION. As it has been demonstrated that the various steroid compounds isolated from the adrenal cortex vary considerably in respect to their ability to maintain the life of adrenalectomized animals, there is

the possibility that the greater potency of the extract from the adrenals of the B_1 deficient dogs may be due merely to a shift from a less active to a more active compound. However, from the results obtained in this investigation it seems most probable that the greater effect is due to an increase in concentration of these compounds in the adrenal cortex. The results from the standardization of the bitterlings showed that increased amounts of an identical extract produced greater elongation of the ovipositors. This would seem to be a concentration effect. That the effect is due to an increased concentration of the steroid compounds is also supported by histological evidence in which an increased lipoid content and a wider distribution of the lipoids throughout the cortex have been described. Finally, it has been shown (Goodsell, 1941) that the hypertrophy of the adrenal gland which occurs in acute vitamin-B_1 deficiency is due to an increase in size of the cortex. The results from this study demonstrate an accompanying increased potency of the steroid fraction. The simplest explanation of these related phenomena seems to be that an increased amount of the active substance has been produced.

SUMMARY

1. The use of the bitterling test as a sensitive biological method for a quantitative determination of the changes in the concentration of the steroid compounds in the adrenal cortex has been described.

2. By the use of this test it has been shown that the concentration of steroid compounds in the adrenal cortex was increased in dogs suffering from acute vitamin-B_1 deficiency.

Acknowledgment is made of indebtedness to Dr. A. J. Carlson, under whose direction this work was carried out, and to Dr. A. W. Martin for his constructive advice and criticisms.

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THE INFLUENCE OF FATS ON THE MOTOR ACTIVITY OF THE PYLORIC SPHINCTER REGION AND ON THE PROCESS OF GASTRIC EVACUATION STUDIED BY THE BALLOON-WATER MANOMETER AND BY THE OPTICAL MANOMETER-FLUOROSCOPIC TECHNICS[1]

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Fats introduced into the lumen of the proximal intestine inhibit the *body* of the stomach of fasting animals (1, 2). Extending these studies, we have employed the tandem balloon method of Meschan and Quigley (3) to make a qualitative study of the effects of fats on the motor activity of the pyloric *sphincter region* (antrum, sphincter, duodenal bulb and distal duodenum). In a second series of experiments, a modification of the optical manometer-fluoroscopic technic of Brody, Werle, Meschan and Quigley (4) was used to measure quantitatively the effects of fats on antral and bulbar pressures. Our recording tips for these studies were placed in the antrum and bulb on either side of the sphincter 18 mm. apart, and were made of soft rubber with metal inserts. The internal diameter of the antral and bulbar tips was respectively 5 and 3.5 mm.

Fasting animals. The test substances at body temperature were introduced into the duodenum of dogs 18 hours post-cibal at the onset of the experiment. The pressure employed for injection (15 cm. of water) just sufficed to overcome the friction in the catheter and cause the material to enter the duodenum slowly but without distending it. Time intervals in this report were measured from the *beginning* of the fat administration period.

Cream having 25 to 30 per cent butter fat and egg yolk with approximately 33 per cent lipids were employed as common examples of naturally emulsified fats. In 75 experiments cream and in 10 experiments egg yolk was administered into the distal duodenum of animals being studied by

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the tandem balloon method. Inhibition of motility occurred in the *entire* sphincter region. This was manifested by a decrease in frequency and amplitude of the recorded waves which was most pronounced in those from the antrum and sphincter, less marked in the bulbar and comparatively slight in the distal duodenal records (fig. 1). With 10 cc. doses of either cream or egg yolk, the inhibition began in 1.5 to 2 min. and became complete in 4 min. in the antrum and sphincter; if inhibition of the bulb became complete, it developed in 7 min. Diminution of tone, as indicated by a decrease in basal levels, usually ran concurrent with the decreased motility,

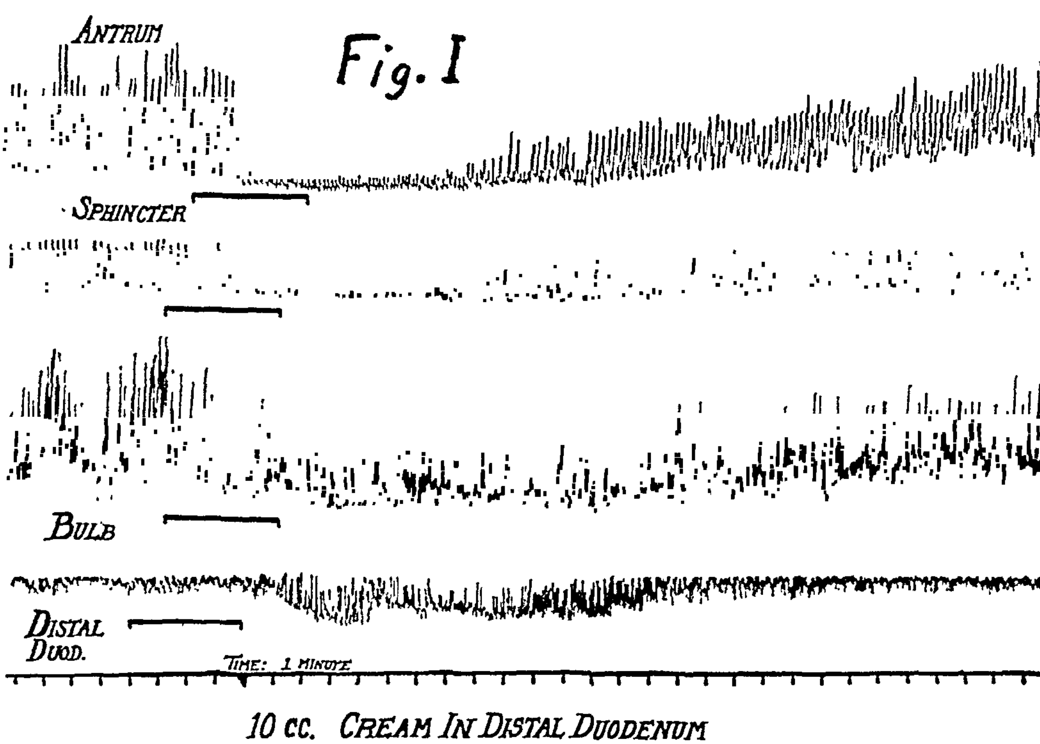

Fig. 1. Action of 10 cc. cream (30 per cent butter fat) introduced into the distal duodenum on the motor activity of the antrum, pyloric sphincter, duodenal bulb and distal duodenum. Balloon-water manometer registration. Time indicated in one minute intervals.

although the minimal tone level usually developed after 10 min. Loss of tone was usually moderate in degree in all four regions, but occasionally the loss was marked in the distal duodenum. Recovery of motility and tone of the entire sphincter region usually began in about 15 min. and was complete in 20 min. Administration of a second injection of 10 cc. of cream 30 min. following the first portion reproduced the previous series of events.

Greater quantities of cream up to 30 cc. prolonged the effect so that complete recovery required as long as 30 min. The effects on the antrum

and sphincter from large volumes of cream were not otherwise altered except that complete inhibition of the bulb and distal duodenum was of more frequent development. Small quantities of cream (2–4 cc.) produced complete inhibition of the antrum and sphincter persisting for 1 to 2 min. The influence on the bulb was much less marked and the distal duodenum was practically unaffected. In an attempt to simulate gastric evacuation of a fatty meal, a series of such small injections was made. They produced similar effects on each occasion and motility in the antrum and sphincter could be kept depressed for periods in excess of 90 min. Recovery occurred shortly after the administration of the last dose.

While recording pressures by the optical manometer-fluoroscopic technic from the antrum and bulb of fasting animals we administered 5 cc. of cream (15 expts.) into the duodenum 3 cm. distal to the sphincter. A

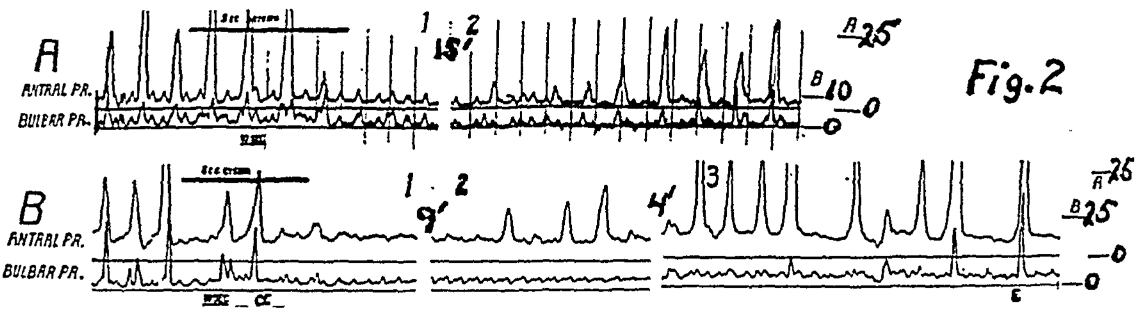

Fig. 2. Records of antral and bulbar pressures obtained by optical manometer technic before and after administering 5 cc. of cream into the proximal duodenum. Time intervals, 10 sec.

A. From a fasting animal. Between *1* and *2* a period of 15 minutes elapsed.

B. From an animal fed cornmeal mush. Period elapsed between *1* and *2* was 9 minutes. Four minutes elapsed between *2* and *3*. Gastric evacuation ceased at *CE*; evacuation returned at *E*.

decrease in the magnitude and frequency of the phasic pressure waves of the antrum and bulb began in about 50 sec. and progressed to complete cessation by 1.5 to 3 min. in the antrum and 3 to 4 min. in the bulb (fig. 2). Occasionally a transient stimulation of the bulb preceded the inhibition. During this excitation interval the frequency and magnitude of the bulbar pressure changes might be doubled and the basal pressure was moderately elevated. This stimulation apparently developed in an effort to empty the bulb and it was followed by a period of bulbar inhibition. Recovery was gradual and complete restoration of pressure waves occurred in the bulb after 8 to 10 min., but only after 13 to 15 min. in the antrum.

When the influence of fat had become maximal, the basal pressure had fallen more in the antrum (2 to 4 cm. water) than in the bulb (usually 0 to 2.5 cm. water). The antral-bulbar basal pressure gradient, normally 3 to 4 cm., fell to between 0.5 and minus 2.5 cm. Three to four cubic

centimeter doses of cream produced results in 12 experiments similar to those obtained with 5 cc., but inhibition of the sphincter region developed slightly later, was less complete and disappeared earlier. Frequently, no alteration in the behavior of the duodenal bulb could be detected with the 2 cc. quantities of cream, but a second administration of the same volume of cream 5 to 8 min. later almost invariably produced a transient inhibition.

Fed animals. Werle et al. (5) employed the fluoroscopic-optical manometer technic on fed animals and demonstrated that gastric evacuation was dependent on propulsive contractions of the distal antrum combined with a positive antral-bulbar basal pressure gradient (evacuation period A) and a positive antral-bulbar phasic pressure gradient (evacuation period B). We have demonstrated that fats in the duodenum of *fasting* animals inhibited the entire sphincter region and lowered the antral-bulbar basal and phasic pressure gradients. This induced us to extend the fluroscopic-optical manometer studies to fed animals in an attempt to explain on a quantitative basis the well established fact that fats retard gastric evacuation.

The influence of cream introduced into the duodenum on the behavior of the sphincter region was investigated in 36 experiments following the administration of food. Twenty-five grams of cornmeal was cooked for one hour; 80 grams $BaSO_4$ were incorporated and the mixture made up to a volume of 500 cc. This material was introduced into the stomach through the gastric cannula and an increase in basal pressures and in frequency and magnitude of the phasic pressure waves of the antrum and bulb was observed.

The food administered *via* the cannula was evacuated without a preliminary pause; frequently evacuation began during the feeding process. The antral and bulbar phasic pressure waves were of increased magnitude and occurred at a frequency of 5 to 6 per minute. The first 2 to 3 peristaltic antral waves gradually increased in maximal pressure. Subsequent waves appeared to divide the antrum into two distinct cavities and almost the entire mass distal to the wave was evacuated at each cycle. The volume evacuated by each cycle was dependent on the depth of the antral wave and the portion of the antrum at which it attained its maximum depth. We estimated that 2 to 4 cc. of gastric contents were discharged at each cycle. Typical basal pressures were: antral, 6 to 8 cm. of water; bulbar, 2 to 4 cm.; maximal phasic pressures were antrum 25 to 90 cm., bulb 20 to 75 cm. Usually these conditions persisted until the stomach was half emptied, then the antral and bulbar basal pressures, the frequency and magnitude of phasic pressure changes and the volume evacuated at each cycle gradually decreased slightly. Our observations are not in accord with the usual report of increasingly powerful contractions as the stomach empties.

After studying the evacuation process and the pressure changes for variable intervals, 5 cc. of cream (25 per cent B.F.) was introduced into the proximal duodenum in 11 experiments. Antral and bulbar motility and pressure waves were completely inhibited and gastric evacuation ceased (figs. 2, 3). Inhibition began in the antrum in 70 sec., in the bulb in 2 min., and became complete in the antrum in 2 to 3.5 min., and usually in the bulb in 3.5 to 4 min. Basal antral and bulbar pressures fell (antral more than bulbar), so the basal pressure gradient varied between plus 1 and minus 2, and regurgitation from the bulb into the antrum was occasionally observed. Reversed peristalsis in the sphincter region was not observed but "to and fro" movement of material between the bulb and the mid-portion of the duodenum was usually exaggerated.

Similar quantities of cream inhibited the sphincter region of fed animals less completely and for shorter intervals than in fasting animals. Complete recovery of the basal pressures, phasic pressure changes and peristaltic contractions occurred in the bulb after 7 to 9 min., and in the antrum about 4 min. later, but gastric evacuation did not begin to return until 15 to 20 min. after the beginning of cream administration. Cream was less effective in inhibiting the sphincter region of fed animals, partly because it was flushed out of the proximal intestine and diluted by the evacuated chyme and also because the presence of food in the stomach augmented the propulsive activity of the antrum.

An augmented contraction of the sphincter was not responsible for the retarded gastric evacuation following cream administration. Studies of the movements of shot sutured to the serosa at either side transversely of the sphincter or records obtained from a miniature balloon placed in the sphincter and arranged to record from an optical manometer showed the sphincter relaxed or, at the most, contracting rhythmically but feebly at this time. This observation is in accord with the results obtained in fasting animals by the tandem balloon technic.

Cream delayed gastric evacuation *chiefly* by depressing propulsive antral peristalsis and thus terminated the development of positive antral-bulbar phasic pressure gradients. The decrease or reversal of the basal antral-bulbar pressure gradient (evacuation phase A) and the increased bulbar activity which sometimes transiently occurred played minor rôles in retarding evacuation.

Recovery of antral contractions usually preceded the reëstablishment of gastric evacuation. The first antral contractions were not of the "propulsive type." Frequently they progressed at a slow rate, were shallow and tended to die out before reaching the sphincter. Such waves did not produce phasic pressure variations. Even when the contraction waves were normal in rate and depth and progressed to the sphincter they failed to occlude the lumen, for the antrum at this time was relaxed to approxi-

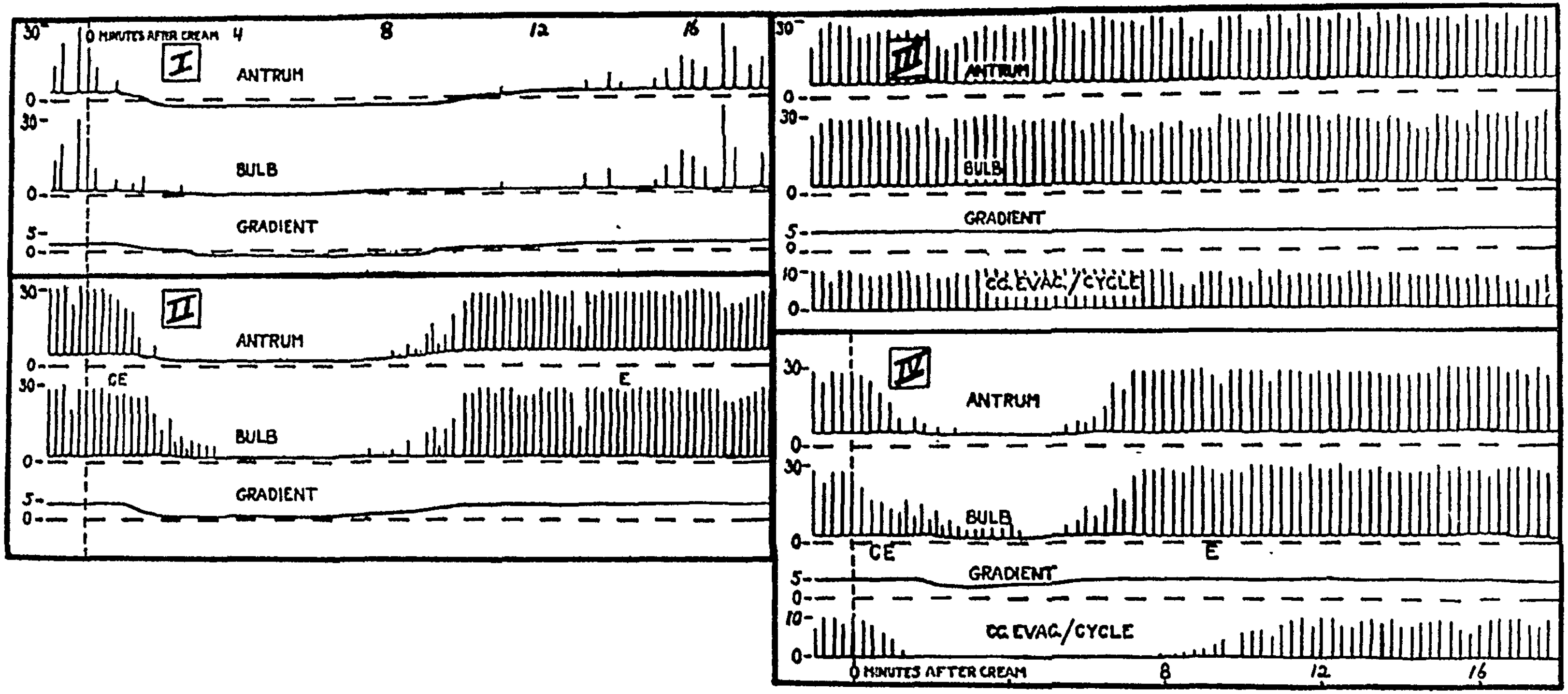

Fig. 3. Graphic representation of pressures obtained with the optical manometer technic from the pyloric antrum and duodenal bulb and the antral-bulbar basal pressure gradient before and after the injection of 5 cc. of cream into the duodenumat 0 minute. Pressures in centimeters water indicated by scale at left side of diagrams. *CE* signifies time of cessation of evacuation, *E* return of evacuation. I, animal fasting for 18 hours. II, animal fed cornmeal mush and $BaSO_4$. III, animal fed cornmeal mush and $BaSO_4$, duodenal drainage, no cream administered. IV, animal fed cornmeal mush and $BaSO_4$, duodenal drainage, 5 cc. cream injected into the duodenum.

mately twice the normal diameter. Thus, the waves produced slight antral phasic pressure changes and the antral contents were not discharged into the duodenum but returned to the body of the stomach. A similar situation was frequently observed during the period which normally elapsed between feeding and the beginning of gastric evacuation.

When 10 cc. quantities of cream were employed (15 expts.) the effects were similar to those obtained with 5 cc. doses, but were more striking and less variable. Complete inhibition of the bulb was sometimes absent with 5 cc. doses, but was always obtained with 10 cc. of cream. The decrease in basal pressure gradient was more constant and more marked; values of minus 2.5 cm. were reached. With small doses of cream (2 to 3 cc.) cessation of gastric evacuation was a more uniform effect than any one of the other phenomena mentioned.

Duodenal drainage. In ten experiments a rubber balloon was introduced into the duodenum about 10 cm. distal to the sphincter. By means of a catheter this balloon was inflated with air to the minimal pressure (20 to 25 cm. water), which largely but not absolutely prevented progress of duodenal contents beyond this point. A second catheter passed through this balloon to permit the introduction of cream into the duodenum about 17 cm. distal to the sphincter. The duodenal balloon and injection tube were inserted 30 min. preceding the experiment proper. A third tube was placed with its open end near the bottom of the duodenal cannula and suction applied so this tube provided duodenal drainage. The volume of duodenal contents collected was periodically measured.

This procedure was designed to study the effect of cream on gastric evacuation and the behavior of the sphincter region when dilution and flushing away of the cream by the chyme was prevented. The results, however, were complicated by the fact that duodenal drainage definitely hastened gastric evacuation. In our experiments the evacuation rate was increased 2 to 4 times. The experiments further differed from the other optical registration experiments since the cream was injected 15 cm. lower in the duodenum and exposure of cream to the action of bile and pancreatic juice was largely prevented.

In the experiments involving duodenal drainage, the fluoroscopic observations showed that the evacuated material passed directly through the bulb and proximal duodenum into the collecting bottle. Frequently the antral contraction forced the chyme without pause as far as the duodenal cannula and contraction of the proximal duodenum was not required for the propulsion. Thus the volume evacuated by each antral peristaltic wave could be measured. After feeding, the first few cycles which evacuated gastric contents discharged small volumes. For the next 3 to 7 min. the volume per cycle was rather irregular but averaged about 10 cc. (occasionally increased to 50 cc.), then gradually declined to a steady state in which

6 to 8 cc. were discharged per cycle until the stomach was almost completely emptied.

Apparently the rate of gastric evacuation normally represents a balance between *a*, an augmenting effect produced by the distension of the antrum and body of the stomach by food; *b* an inhibitory influence produced by the presence of the evacuated material in the intestine, acting in part through mechanical (nonspecific) influences and partly through chemical (specific) factors. Elimination of factor *b* in these experiments releases the evacuating power of the stomach from an important fraction of the intestinal regulation.

Introduction of 5 cc. of cream into the duodenum of fed animals provided with duodenal drainage produced results similar to those obtained from similar doses without duodenal drainage (fig. 3). However, the effects were slower in development, the suppression of phasic pressure waves (especially of the bulb) and gastric evacuation was less complete and of shorter duration in the animals with drainage. As in the experiments without duodenal drainage, the first antral peristaltic waves to return after cream inhibition failed to expel the antral contents into the intestine. Instead, this material usually returned to the body of the stomach for the peristaltic waves did not sufficiently occlude the antral lumen to prevent a retrograde axial stream. Four cubic centimeters was the minimal quantity of cream which was even transiently effective in the drainage experiments.

Gastric distention by a balloon. Stretching the stomach may *per se* produce some of the effects noted from feeding. We distended the body of the stomach of fasting animals with a large balloon and again determined the modification in the activity of the sphincter region and the effectiveness of fat administration. No significant alteration in the activity of the pyloric region was noted with 100 to 150 cc. of air in the ballon. When the distending volume was 500 cc. of air, the activity of the pyloric region approached but did not equal that obtained by administering *via* the cannula 300 cc. of food. Thus, distention of the body of the stomach is one of the factors responsible for the difference in sphincter region activity characteristic of the fed and fasting states. Food stimulates the sphincter region more than a balloon distended to an equal volume. This may be due to the fact that the food, unlike the balloon, enters the sphincter region. Confirming this is the observation that the tandem balloons in the sphincter region gave results from fasting dogs which resembled those obtained by optical registration from fed rather than fasting animals. Apparently, the moderate distention of the sphincter region augmented the motility in that region.

In four experiments with the body of the stomach of fasting animals distended by 500 cc. of air, the administration of 5 cc. of cream into the

proximal duodenum inhibited the sphincter region in a manner similar to the characteristic response observed in the fasting animal whose stomach was not distended. However, the cream inhibition was slightly less marked in the animal with a distended stomach; the period of complete antral inhibition, for example, being 1 to 2 min. shorter.

SUMMARY

In studies made with the tandem balloon method the introduction of fats into the duodenum of fasting dogs inhibited the motility of the pyloric antrum, sphincter and duodenal bulb. A quantitative study of the pressure relations in the pyloric sphincter region combined with a visualization of motility and propulsion was obtained by the optical manometer-fluoroscopic technic. Employing this method, it was again shown that cream in the duodenum inhibited the pyloric region and further proved that cream decreased or reversed the antral-bulbar basal and phasic pressure gradients. The action of cream was most pronounced in fasting animals, then in order of decreasing effectiveness, fasting animals with the stomach distended by a large balloon, fed animals, fed animals with duodenal drainage. Fats retarded gastric evacuation chiefly by decreasing antral propulsive peristalsis. Sphincter spasm was not involved; on the contrary, evacuation was retarded in spite of sphincteric relaxation. The rate of gastric evacuation represents a balance between *a*, the augmenting effect produced by a distention of the antrum and body of the stomach by food, and *b*, the inhibitory influence produced by the presence of chyme in the intestine, acting in part through mechanical (nonspecific) influences and partly through chemical (specific) factors. In experiments involving duodenal drainage, factor *b* was largely eliminated, thus the evacuating mechanism of the stomach was released from an important fraction of the intestinal regulation and the evacuation rate was augmented.

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THE INFLUENCE OF ESTRADIOL ON THE SECRETION OF GONADOTROPIC HORMONE IN ADULT PARABIOTIC RATS[1]

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We have previously reported that the ovaries of a normal immature female parabiotic rat undergo hypertrophy following gonadectomy of its partner, and that the daily injection of relatively small quantities of estrogen and somewhat larger quantities of androgen into the castrated partner of such pairs prevents ovarian enlargement in the normal animal (1–5). These results were interpreted to mean that the estrogen or androgen prevented the hypersecretion of gonadotropic hormone from the pituitary gland of the castrated rat which occurred following gonadectomy.

Using adult parabiotic rats, Hill (6, 7) and Witschi and Levine (8) demonstrated that the pituitary gland of the partner which was gonadectomized underwent hypersecretion, for the normal female partner developed a condition of continuous estrus. Because it was possible to prevent hypersecretion of gonadotropic hormone in immature pairs by the injection of estrogen, it became of interest to determine whether estradiol[2] acted similarly in adult pairs which had developed a continuous vaginal estrus.

Materials and methods. Female littermate rats weighing 70 grams or more were united in parabiosis at 31 to 33 days of age according to the method of Bunster and Meyer (9), except that metal skin clips were used instead of silk sutures in closing the skin incisions. Ether anesthesia was used and sterile precautions were taken during the operation. The right-hand partner was ovariectomized at the time of parabiotic union. Following the opening of the vagina of the normal partner, which usually occurred 7 to 8 days following parabiotic union, the vaginal smear was followed to determine the time at which continuous estrus appeared. After a period of 25 to 30 days of continuous estrus, injections of 1 γ of estradiol per day were made into the ovariectomized partner. The amount of

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hormone injected each day was contained in 0.05 cc. of corn oil. At varying intervals the dosage of estradiol was increased to 2, 3 or 4 γ per day in some of the pairs, as is indicated in the figure. The ovaries of the normal partner were examined under a binocular dissecting microscope during laparotomies which were performed before or during the estrogen treatment. In pairs 3, 5 and 7 one ovary was removed for histological study before the estradiol injections were begun. In pairs 5 and 6 the ovariectomized partner was hypophysectomized after the normal partner had shown a second extended period of continuous estrus following withdrawal of the initial estrogen treatment.

Records of representative pairs are presented in figure 1. It should be emphasized that the figure presents the vaginal smear record of the normal female partner, and that the injections of estradiol were always made into the ovariectomized partner. The notations concerning estrogen treatment of the pairs refer to the time at which injections were either begun or discontinued. Day 0 is the first day of continuous estrus, and all successive days are dated from this time. Laparotomies of the normal partner are indicated by the letter *L*. The injections of the luteinizing hormone, *LH*, were made into the ovariectomized partner of pair 7.

Results and discussion. The data were obtained from 14 pairs of rats, and of these three were treated a second time with estradiol, so the total number of experiments was 17. Records of 7 pairs are included in the figure.

The vaginal smear records show that following injections of estradiol into the ovariectomized partner, the continuous estrus of the normal partner was replaced by a temporary diestrus. Usually 1 γ of estradiol per day was sufficient to cause the vaginal change, although the dosage was raised to 2 γ per day in one pair before this change occurred. Laparotomies performed before estrogen injections were made showed that the ovaries were composed of numerous large follicles, some of which were cystic. These observations were confirmed by histological sections of ovaries removed at this time.

The diestrus which followed the estradiol injections varied from 1 to 20 days, the average duration being 10 days. The ovaries of the normal partner of all the pairs examined except one (not included in figure), contained follicles which now appeared to be smaller than those present at the beginning of the estrogen treatment. The exceptional pair had three corpora lutea in one ovary. The diestrus of the normal female was followed by estrous cycles, which were often somewhat irregular in some of the pairs. However, by increasing the dosage of estradiol to 2 γ per day, they tended to become more regular (pairs 2, 3 and 5). It was necessary to increase the dosage to 3 γ per day in one pair before regular cycles were obtained. The estrous cycles of the normal female persisted as long as the ovariectomized rat was injected with estradiol. Laparotomy of the normal

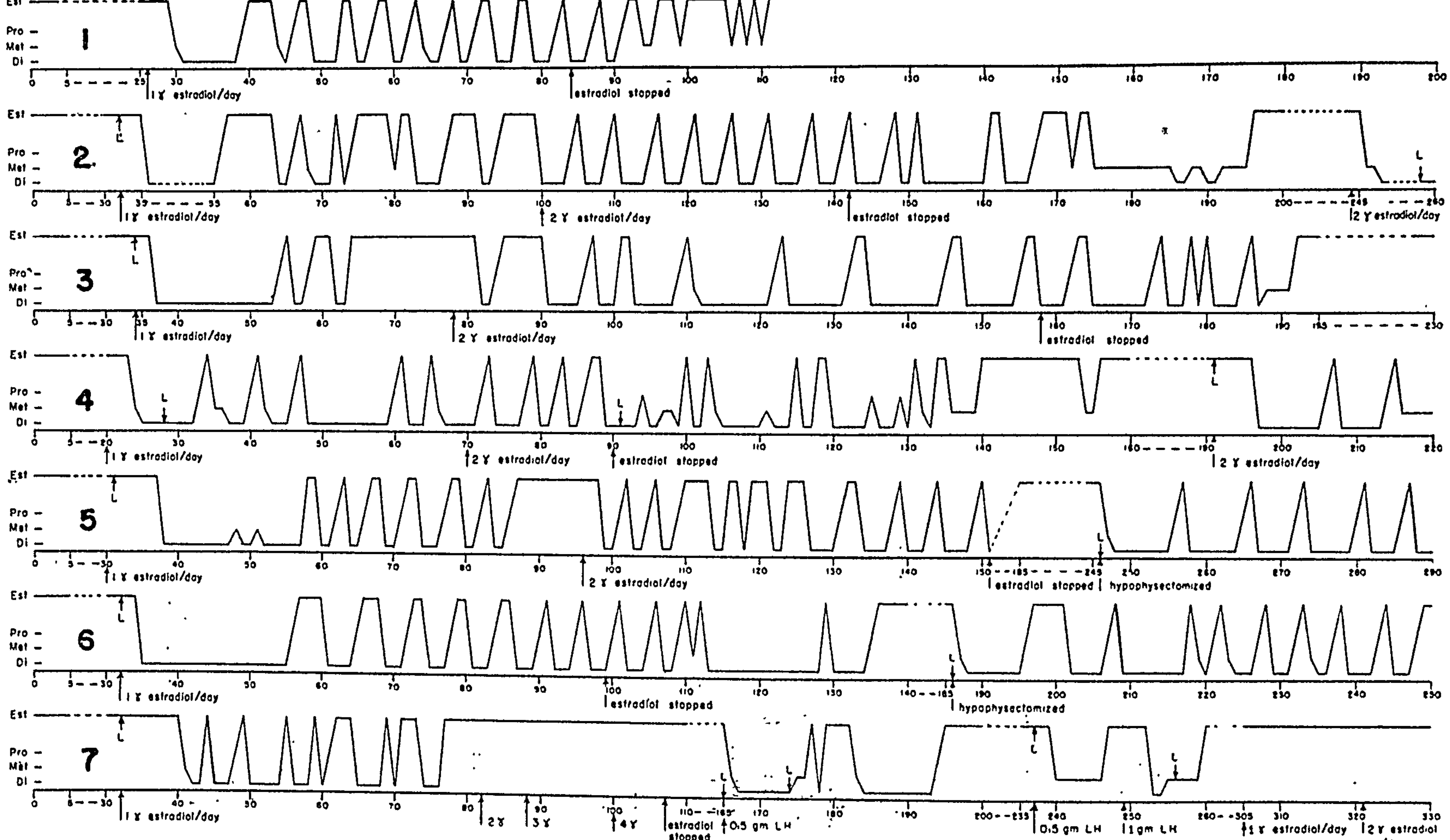

Fig. 1. The vaginal smear record of the normal partner is represented. Day O is the first day of continuous estrus of the normal parabiont following ovariectomy of the other. The ovariectomized partner received the injections. The ovariectomized rat of pairs 5 and 6 was hypophysectomized. Laparotomy of the normal partner is represented by the letter *L*.

partner of pair 4 during this period showed that the ovaries were composed of follicles and corpora lutea. Following cessation of the estrogen treatment, several normal estrous cycles usually occurred, after which the cycles became irregular and finally continuous estrus reappeared.

The ovariectomized partner of pairs 5 and 6 was hypophysectomized after an extended period of continuous estrus which followed the withdrawal of the initial estradiol treatment. Here, as during the estradiol injections, the continuous estrus of the normal female was replaced by a diestrus, after which normal cycles appeared. Pair 5 had 16 regular cycles, and pair 6 had 17 after hypophysectomy and before autopsy.

Comparison of the results following the injection of estradiol with those found after hypophysectomy suggests that the estradiol prevented the secretion of gonadotropic hormone from the pituitary gland of the ovariectomized rat. Moreover, the administration of the proper amount of estradiol was apparently as effective in bringing about regular estrous cycles in the normal partner as was the removal of the pituitary gland. Pair 7 appears to be an exception to this since two of the three periods of estradiol treatment were not effective in changing the continuous estrus to diestrus. During the first treatment, a short period of continuous estrus appeared while 1 γ of estradiol per day was being injected. Consequently the dosage of estradiol was increased to 2, 3 and finally 4 γ per day, but these amounts were ineffective in changing the vaginal smear. Also, at a subsequent time, injections of 1 and 2 γ of estradiol per day were made, without changing the type of vaginal smear. From these results it appears that the pituitary gland of the ovariectomized rat of this particular pair became refractory to the estrogen treatment.

Injections of LH were made into the ovariectomized partner of pair 7 to determine whether this substance could cross to the normal parabiont and luteinize the follicles. After each of 3 injections of LH, the vaginal smear of the normal partner changed from continuous estrus to diestrus and corpora lutea were found in the ovaries (see figure). It will be noted that the diestrus which occurred after each injection was short and was followed by periods of prolonged estrus. The results of this experiment demonstrate that the ovaries of the rat in continuous estrus are sensitive to LH and that LH can be transferred from one parabiont to the other. This conclusion is of importance in explaining the cause of the period of prolonged diestrus which followed immediately after the injection of estrogen into the ovariectomized partner. In all the pairs examined during this period except one, the ovaries were found to be small and devoid of corpora lutea. These results and those obtained by injecting LH into the ovariectomized partner of pair 7, provide evidence for the concept that the injection of estrogen in the amounts used in these experiments, *does not* bring about the release of sufficient quantities of LH from the pituitary gland of the ovariectomized partner to luteinize the ovaries of the normal

parabiont. Hohlweg (10) and Fevold, Hisaw and Greep (11), however, have shown that estrogen causes the formation of corpora lutea in single normal immature and mature rats. This luteinization of the ovaries was attributed to a release of LH from the pituitary gland by the injected estrogen.

It seems that the diestrus of the normal rat, which followed the injections of estradiol into the ovariectomized parabiont, can be explained best on the basis that the injections of estradiol prevented the secretion of gonadotropic hormone from the pituitary gland of the ovariectomized rat. It is also suggested that the length of the diestrus represents the time required for the pituitary gland of the normal partner to recover from the inhibiting influence of the estrogen produced by its own ovaries during the time that they were continuously stimulated by the gonadotropic hormone from the castrated partner. Therefore, it would seem that the appearance of estrous cycles in the normal animal after the period of prolonged diestrus represents the resumption of cyclic activity by the pituitary gland and ovaries of this rat.

Evidence that there was very little, if any, transfer of the injected estrogen from the ovariectomized partner to the normal, is furnished by the occurrence of normal estrous cycles in the latter during the period of injection. If the injected estrogen were present, it would no doubt have interfered with the estrous cycles of the normal partner, for it was determined in a group of normal female rats that the injection of 1 γ of estradiol per day was sufficient to cause continuous vaginal estrus. Therefore, it seems unlikely that the estrogen passed from the injected to the normal partner in amounts sufficient to interfere with the normal cyclic activity of the pituitary gland and ovaries.

One pair, which is not included in the figure, was not in continuous estrus when injections of estradiol were begun, and the subsequent cycles were very irregular and appeared to be no different from those obtained before beginning the injections. Laparotomy 29 days following the beginning of injections showed that there were many corpora lutea in the ovaries of the normal partner. Subsequent treatment with 2 and 3 γ per day likewise did not produce regular cycles. Furthermore, the normal partner did not remain in continuous estrus after the injections of estradiol were stopped.

Hill (6) reported that usually 3 months' time was required following ovariectomy before continuous estrus was attained. Our rats required considerably less time to reach this stage, since the average time was 39 days. Kawashima also noted a shorter period of time than that required by the rats used by Hill (cited by Hill).

In immature female-female pairs, it was found that 0.025 γ of estradiol per day was required to prevent hypersecretion of the gonadotropic complex following ovariectomy (4). In mature pairs, from 1 to 3 γ of estradiol

per day have been found to be necessary. However, this difference in the two studies should be pointed out, namely, that the estrogen treatment was begun immediately following gonadectomy in the immature pairs, whereas it was begun after the hypersecretion had become well established in the adult pairs. In the former case there was probably very little gonadotropic hormone being secreted by the pituitary gland, whereas in the latter, gonadotropic hormone secretion was at a very high level. Although no attempt was made to determine the minimum amount of estradiol required to prevent castration hypersecretion in the adult pairs, it would seem that they probably require more estrogen to prevent hypersecretion of the gonadotropic complex than do immature parabiotic rats.

SUMMARY AND CONCLUSIONS

Continuous vaginal estrus was obtained in the normal partner of female parabiotic rats at an average of 39 days following ovariectomy of the other partner.

After an extended period of continuous estrus, injections of estradiol were made into the ovariectomized rat. As a result of these injections, the continuous vaginal estrus of the normal partner was replaced by a diestrus, which persisted an average of 10 days. Normal estrous cycles then appeared and usually persisted as long as the injections of estradiol were continued. After stopping the injections, the estrous cycles became irregular and finally continuous estrus reappeared. This treatment was repeated several times in some of the pairs with the same results.

The ovariectomized partner of pairs in continuous estrus was hypophysectomized, and, as during the estradiol treatment, the continuous estrus of the normal partner was replaced by a diestrus. Normal cycles then appeared and persisted until the rats were autopsied.

Because the results following the injection of estradiol into the ovariectomized partner were similar to those following hypophysectomy, it is concluded that estrogen prevents the hypersecretion of gonadotropic hormone by the pituitary gland of adult ovariectomized rats.

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THE MECHANISM OF DEFLATION HYPEREMIA IN THE INTESTINE

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The most consistent, and one of the most striking, effects of low-pressure distention of the dog's small intestine (below 40 mm. Hg lumen pressure) is the period of increased arterial flow beginning simultaneously with the deflation and lasting 20 to 30 seconds or longer. This period has been described as the third phase of the total response (Lawson and Chumley, 1940). In the present report it will be called deflation hyperemia. As a prominent spike of augmented flow it either terminates the response or passes more or less abruptly into the terminal fourth and fifth phases, with which this report is not concerned.

Deflation hyperemia is not materially affected by mesenteric denervation of the loop, yet is abolished or markedly reduced by local treatment of the loop with cocaine. It is not seen following low-pressure inflations if the loop is encased in plaster to prevent enlargement. On the basis of these and similar data it has been suggested that it represents the persistence for a short time beyond the distention period of vasodilatation (or an extravascular resistance-lowering mechanism) set up through the peripheral nervous apparatus by stretch of the gut walls. This interpretation of deflation hyperemia, although compatible with all the data available, cannot be regarded as final without further study. Treating the loop with cocaine or encasing it in plaster would not only abolish hyperemia from the mechanism postulated, but might also, through an alteration in the mechanics of the loop, modify all phases of a response which is wholly mechanical, or primarily mechanical, with secondary non-nervous phases.

The present report considers two non-nervous mechanisms which might account for an increased arterial flow into the intestine immediately following distention, in an attempt to evaluate their contribution to deflation hyperemia: 1, the rapid filling under a steepened pressure gradient of vessels the volume of whose contents has been diminished during the distention; 2, post-ischemic vasodilatation (reactive hyperemia) in portions of the intestine deprived of blood by the distention.

The first mechanism, a filling phenomenon, has been held responsible by Rössler and Pascual (1932) for an overshoot in coronary flow which they

recorded at the beginning of diastole, and by Anrep, Blalock and Samaan (1934) for a similar phenomenon observed in skeletal muscle at the termination of a tetanus. As described for these tissues it is of very brief duration (less than 0.2 sec. for skeletal muscle). Its duration in the intestine during deflation would depend upon the rate of inflow and the blood volume to be replenished as well as upon the rate of withdrawal of the compressing force. In the absence of data on these values it is unwise to conclude that this factor could not account for deflation hyperemia lasting usually 20 to 30 seconds, and sometimes longer than 1 minute.

The fact that deflation hyperemia bears no relationship to the gross flow deficit incurred during the distention, and is observed even when there has been an increase in flow (Lawson and Chumley, 1940b), does not exclude the mechanism of reactive hyperemia, since redistribution of blood in the distended loop may leave some regions ischemic, the local ischemia being masked by an increased flow in other regions. In fact, unless the resistance-lowering mechanism which is set up by stretch acts to maintain the flow through the compressed regions, masked ischemia would be expected to develop in these regions during the distention, to cause reactive hyperemia on deflation.

A third possibility, that the overshooting on deflation is due to properties of the flow-meter (Lawson, 1940a), has not merited separate treatment, since no overshooting is recorded when arteries drained through cannulas are clamped or released or when the flow-meter installation is tested with suddenly changed flows in artificial systems, and since deflation hyperemia with identical features has been recorded with other types of differential manometers (independent mercury manometers—see fig. 1; inverted U-manometers—see Lawson and Chumley, 1940).

Methods and results. Short loops of ileum were prepared in barbitalized dogs as already described. The distentions employed were similar in duration (usually less than 2 min.) and magnitude (usually less than 40 mm. Hg distending pressure) to those of the previous study. In most of the data presented here the metal bellows manometer (Lawson, 1940) was used to read the pressure fall across the applied constriction in the mesenteric artery. Recorded pressure differences were converted into volume flow of blood by calibration *in situ* at the close of each experiment. The pressure difference, plotted as zero, for zero flow into the loop was obtained by clamping the artery in the mesenteric pedicle of the loop (below the constriction and the lower cannula). With this clamp in place to stop flow into the loop, a second artery below the constriction or a side arm on the distal cannula was opened and flow through this outlet regulated with a screw clamp to produce manometer deflections within the limits observed in the experiment. Three to five such deflections were plotted against their corresponding measured flows to obtain a calibration curve

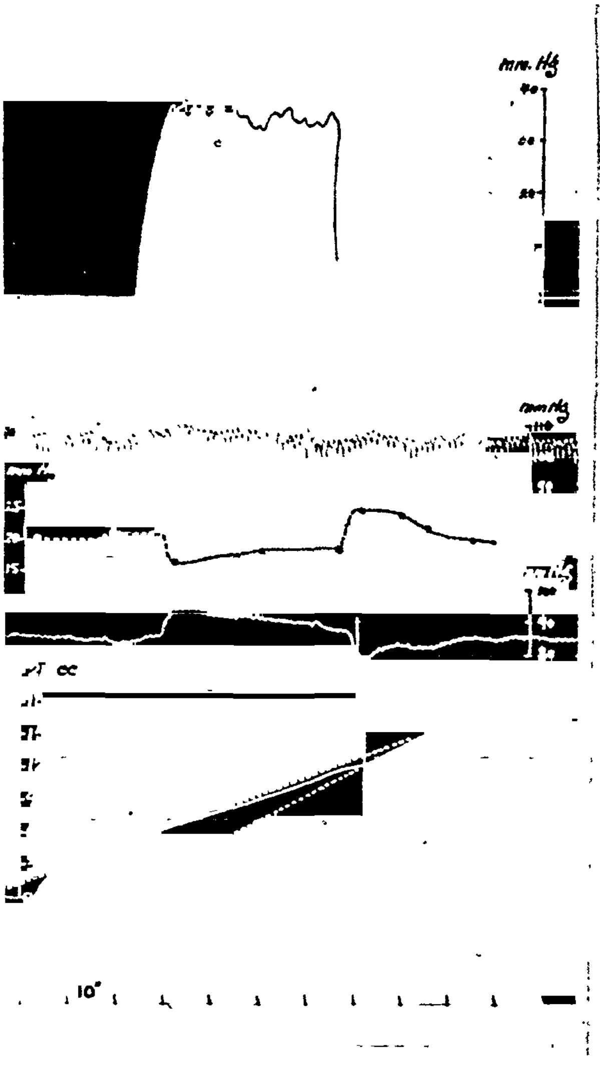

Fig. 1

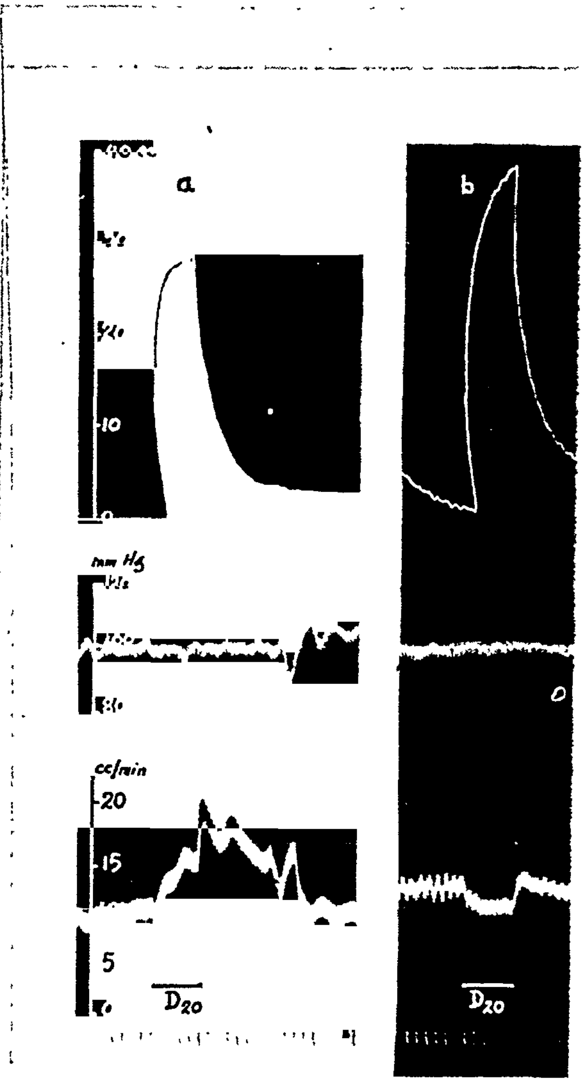

Fig. 2

Fig. 1. The uppermost record is a tracing of distention pressure. The second is a tracing of carotid pressure, the fourth a tracing of mesenteric arterial pressure below the constricting clamp, recorded by independent mercury manometers. A qualitative flow record comparable to those in the other figures is constructed by plotting (third tracing) the pressure difference at the points marked by circles, between the two manometers. The lowermost record is a tracing of venous flow, made by recording air displacement from a flask receiving the outflow from the cut vein. The upper dotted line drawn on the outflow record is a continuation of the control rate of flow, the lower dotted line a continuation of the increased rate of flow following deflation. Corrected simultaneous ordinates are drawn through the venous outflow record at the point where rate of flow begins to exceed the control, and through the record of mesenteric arterial pressure. Time in 10-second intervals.

Fig. 2. Uppermost tracing records the volume of fluid accepted by the loop from the distention reservoir. The second tracing is a record of carotid pressure, the lowermost a record of arterial flow into loop (mesenteric denervation, weight 34 grams). The signals mark distention at a pressure of 20 cm. water. Time in 10-second and 1-minute intervals. *a* shows the response before, and *b* after 10 cc. 1 per cent cocaine hydrochloride had been substituted for an equal volume of loop fluid. (Loop distended with 0.9 per cent NaCl solution directly from reservoir, without balloon.)

for each experiment. The plotted data sometimes lay on a straight line, but usually on a smooth curve convex toward the flow axis. Extrapolation on the calibration curve to the true zero pressure axis usually showed a flow past the constriction of 0.5 to 2.0 cc. per minute with the loop clamped off. This small uncalibrated flow never amounted to more than 10 per cent. of the total flow past the constriction, usually considerably less. It goes to the pedicle central to the occluding clamp, and to sessile lymph nodes along the axis of the superior mesenteric artery peripheral to the constriction. That it remains fairly steady through procedures such as are employed in the present study is suggested by the relatively fixed level of residual flow recorded when flow to the loop is stopped (fig. 4).

Expressed as volume flow per minute per 100 grams of postmortem intestinal weight, the average control flow into six innervated loops was 36 cc., with extremes of 25 and 48 cc.; into eight loops with mesenteric denervation, 42 cc., with extremes of 30 and 55 cc. These values agree reasonably well with the value of 31 cc. per 100 grams per minute obtained by Burton-Opitz (1908) for venous flow from innervated small intestine under similar experimental conditions.

The rôle of the filling mechanism. That time is required for the suddenly decompressed vascular bed to fill up was suggested by Burton-Opitz (1908) in explanation of a brief stoppage of venous flow from the intestine during deflation. The stoppage of outflow during the first portion of deflation has been repeatedly confirmed in the present investigation (Lawson and Chumley, 1940). If the increased inflow which begins at the same time is due altogether to low pressure in the decompressed vascular segments, it should terminate as soon as pressure in these segments has returned to normal. Other things being equal, changes in venous outflow from the loop may be taken as an index to pressure changes in the segments which are being compressed and decompressed by distention and subsequent deflation. Under these conditions it may be concluded that pressure in peripheral segments during decompression has returned to its control value as soon as venous flow recovers its control value.

In a total of twelve satisfactory observations on four dogs, venous outflow recovered or exceeded its control value within 2 to 8 seconds after the beginning of deflation. Simultaneous records of inflow and outflow usually showed that the peak of deflation hyperemia was reached later than this (fig. 1). From 75 to approximately 95 per cent of the total excess flow (area between control and hyperemic flow levels) always occurred more than 10 seconds after the beginning of deflation, at a time when venous flow, on the basis of these data, should have recovered or exceeded its control value. During the greater part of the period of deflation hyperemia both inflow and outflow are increased.

If filling of the decompressed vascular segments is solely responsible for

deflation hyperemia, the excess volume of flow on deflation should equal the volume reduction in the vascular bed during distention. An attempt to obtain data on the reduction in vascular volume in the distended loop by gravimetric oncometry (Lawson, 1940b) gave equivocal results. The loop sometimes increased, and sometimes decreased in weight, the changes usually being small. Data obtained by more conventional methods are presented in table 1. The volume increase in the lumen of the loop (ΔV_i) was recorded by the method already described (Lawson and Chumley,

TABLE 1

For explanation see text

DOG NUMBER	DISTENDING PRESSURE	$\Delta V_e - \Delta V_i$ (cc.)			DEFLATION HYPEREMIA
		10″	20″	Terminal	
	cm. aq.				*cc.*
1	20	+0.2	±0.0	+0.5	0.05
	30	−0.5	−0.6	−0.7	0.33
	30	−0.3	−0.9	±0.0	0.20
	30	+0.4	+0.8	+0.8	0.25
	40	−0.2	−0.5	−0.9	1.50
	40	±0.0	−0.9	−0.9	1.00
2	20	−0.2		−0.2	0.50
	30	+0.6		−0.4	0.35
	30	−0.2		−0.7	0.80
	30	−0.2		−1.2	0.50
	40	−1.2		−2.8	0.60
	50	−0.1		−2.1	0.70
	60	+0.1		−2.1	0.90
	70	−0.6		−3.2	1.90
	70	−1.1		−3.9	2.40
	80	−0.4		−3.9	3.30

Note: In dog 1 $\Delta V_e - \Delta V_i$ persisted as a positive value (increase in wall volume) and in dog 2 as a negative value (decrease in wall volume) for 1 to 3 minutes after deflation.

1940b). The external volume increase of the loop (ΔV_e) was determined simultaneously by placing the loop in the usual type of oncometry chamber, connected with a volume recorder. The value of $\Delta V_e - \Delta V_i$ represents the change in the volume delimited by the distending balloon in the interior of the loop and the peritoneal covering of the loop and pedicle on the outside. The erratic values which are shown in the table, in agreement with those obtained by the gravimetric method, suggest that extravascular fluids, either in the lumen or in the gut wall, may change their volume unpredictably during distention to mask the change in vascular volume. The initial volume change, read at the end of 10 seconds following infla-

tion, is probably more nearly a pure vascular volume change since the extravascular changes would be expected to be somewhat slower. The peak of inflow reduction has usually been passed at the end of 10 seconds' distention, and the enlargement of the loop is almost complete. The emptying of the vascular bed under compression is probably therefore complete. Examination of the table shows that in only three out of a total of sixteen distentions was the volume change at this time equal to more than 50 per cent of the excess volume flow during deflation; in five cases the volume change at this time was in the wrong direction and therefor could account for none of the deflation hyperemia, and in the remaining eight distentions the volume change could account for only 12 to 44 per cent of the hyperemia.

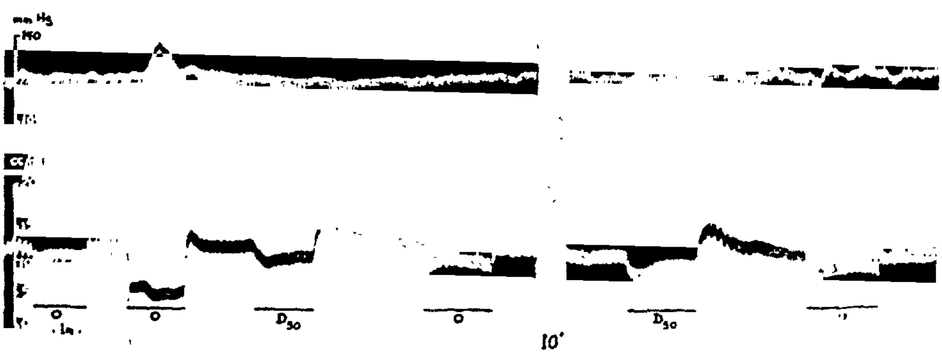

Fig. 3. Upper record is carotid pressure (mercury manometer), lower record arterial flow into loop (weight 30 grams). During the signals marked *O* the artery supplying the loop was partially occluded. During the signals marked *D* the loop was distended at a pressure of 30 cm. water. Timer marks intervals of 10 seconds and 1 minute.

The rôle of reactive hyperemia. It was assumed for the purposes of the present study that the post-ischemic behavior of the loop as a whole following a period of arterial occlusion can be taken as a model of the behavior of any tissue in the loop which might be subjected to ischemia during distention. Complete arterial occlusions were produced with a bull-dog artery clamp, or with a modified Goldblatt clamp, and partial occlusions with the latter, applied in the mesenteric pedicle of the loop. The artery was stripped of gross nerve fibres at the site of the clamp.

When the flow reduction produced by a distention was duplicated by partial arterial occlusion, the hyperemia following the distention was always the greater (fig. 3). The gradual increase in flow during partial occlusion

shown in the figure is typical of the partial, but not of the complete occlusions (see fig. 6). It resembles the gradual decrease in the pressure difference which is observed for 3 to 5 minutes after application of this type of clamp as the flow-meter constriction, and probably represents loss of tone in the segment of artery within the clamp. In most animals flow had to be reduced 30 to 50 per cent by arterial occlusion in order to elicit hyperemia. Gross flow reductions of this magnitude were never obtained

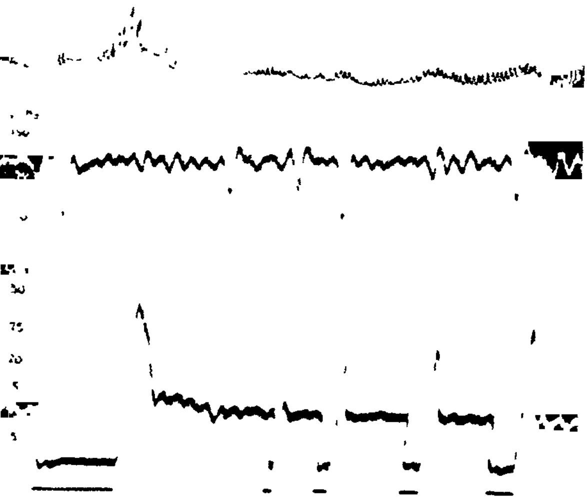

Fig. 4

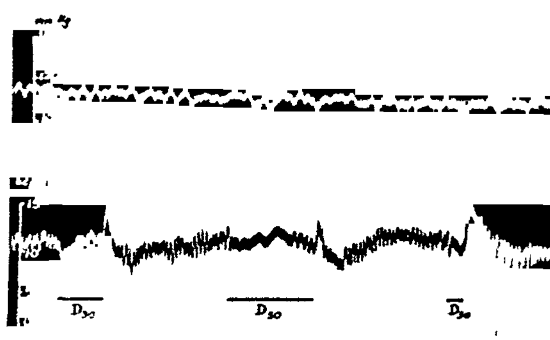

Fig. 5

Fig. 4. Uppermost tracing is a record of loop motility. Second tracing is a record of carotid pressure. Third tracing is arterial flow into loop (mesenteric denervation, weight 35 grams). The signals mark complete stoppage of flow to the loop by application of a bull-dog clamp to its artery. Time in intervals of 10 seconds and 1 minute.

Fig. 5. Upper tracing is a record of carotid pressure. Lower record is arterial flow into loop (mesenteric denervation, weight 27 grams). The signals mark loop distentions at a pressure of 30 cm. water. Timer marks intervals of 10 seconds and 1 minute. The small regular waves in flow at a rhythm of 8 to 10 per minute which appear in this record are usually synchronous with recorded rhythmic intestinal movements.

with the low-pressure distentions of this and the previous report. If all tissues in the loop share equally the reduced flow during partial arterial occlusion, the threshold flow reduction for all tissues, for the production of reactive hyperemia, must lie within or above this range. If, then, deflation hyperemia is merely reactive hyperemia in portions of the loop which have suffered supraliminal flow reduction during the distention, the flow through these portions must have been reduced at least 30 to 50 per cent by the distention.

Complete arterial occlusions of shorter duration than 20 seconds usually were followed by no increased flow. Prolonging the duration of the occlusion beyond this up to 2 minutes or longer increased the height and the area of the resulting hyperemia (maximum rate of flow and total excess flow) (fig. 4). In contrast, neither the height nor the area of deflation hyperemia increased with the duration of distention, provided the distention was sufficiently prolonged to permit fairly complete enlargement of the loop (fig. 5). In four dogs in which a study of this point was made, distentions prolonged to 2 minutes or longer were followed by smaller deflation hyperemias than distentions lasting 20 to 30 seconds.

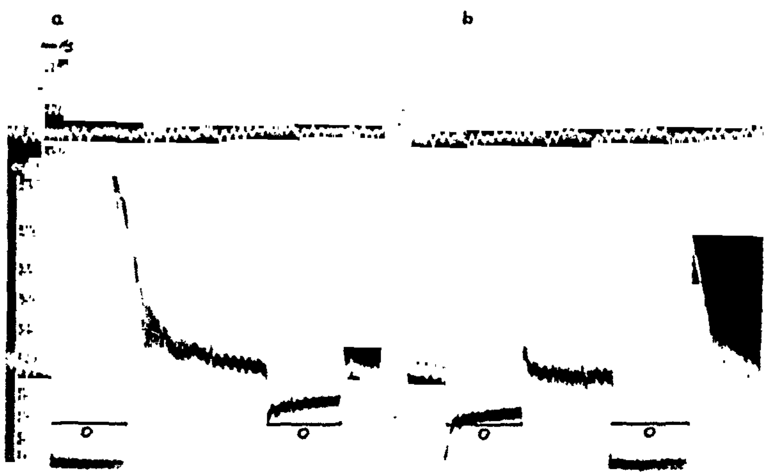

Fig. 6. Upper tracing is a record of carotid pressure. Lower record is arterial flow into loop (mesenteric denervation, weight 35 grams). The signals mark partial or complete stoppage of flow into loop with Goldblatt clamp applied to its artery. Time in 10-second intervals. *a* shows reactive hyperemia before, and *b*, after introduction of 10 cc. 1 per cent cocaine hydrochloride into lumen of loop. The flow deficits before and after cocaine are nearly equal.

The practically complete abolition of deflation hyperemia by local application of cocaine is shown in figure 2. With low-pressure distentions which cause an increase in flow over the control during the distention period, cocaine abolishes both the hyperemia during distention and the deflation hyperemia, as shown in the figure. In comparison, reactive hyperemia following arterial occlusion was relatively little affected by cocaine (fig. 6). As might be expected from any vasoconstrictor agent, the absolute values of reactive hyperemia in terms of the maximum rate of flow and the volume of flow excess were usually somewhat reduced by cocaine. In two animals an attempt was made to obtain a quantitative comparison of the effect of cocaine on the two types of hyperemia, by expressing all responses as percentage changes from the control rates of flow.

In one animal cocaine reduced deflation hyperemia 85 per cent, reactive hyperemia 57 per cent; in the other cocaine reduced deflation hyperemia 50 per cent, and increased reactive hyperemia 45 per cent (all figures are based on the average of 2 to 6 trials before and after cocaine).

DISCUSSION. The reduction in vascular volume during distention should equal the excess volume flow on deflation only if no change other than compression and decompression occurs in the vascular bed. If the data afford even a rough approximation (and not even this can be claimed with certainty for them) of the amount of blood driven out of the peripheral segments during distention, it is clear that deflation hyperemia usually accomplishes much more than just the return of this volume. Insofar as the data are defensible, they show that the "filling" mechanism could account usually for no more than 45 per cent of the deflation hyperemia. Whether, as the data suggest, vascular volume may increase during distention, will have to be determined with more suitable methods.

The less questionable data on the time required for the decompressed vascular bed to fill up to its normal pressure levels and so restore venous flow suggest that no more than 25 per cent, and usually considerably less, of the deflation hyperemia could be due to this mechanism. This is probably a more accurate evaluation.

On the basis of these data it is probably justifiable to conclude that 75 per cent or more of the excess flow during deflation is the result of some other mechanism. That the other mechanism, which is largely responsible, is not reactive hyperemia, is suggested by its abolition with cocaine, and by its failure to increase with increasing duration of the inciting cause. It seems clear that in the distended cocainized loop the reduction in flow is not sufficient, in any tissue, to cause reactive hyperemia. Data to be published elsewhere show that the hyperemia following excessive distentions under pressures approaching arterial pressure, is not abolished or as greatly reduced by cocaine. It is likely that reactive hyperemia contributes to the excess flow on deflation only after such excessive distentions. The decrease of deflation hyperemia with increasing duration of the distention requires further study before an explanation can be offered.

These data, by excluding the two mechanisms studied from significant participation, support the interpretation of deflation hyperemia offered in the earlier report.

SUMMARY AND CONCLUSIONS

The increased arterial flow into loops of small intestine following periods of low-pressure distention (deflation hyperemia) is associated except for the first few seconds following deflation with an augmented venous flow, and usually is considerably greater in volume than the demonstrable volume

reduction of the gut walls during the distention. It is concluded that only a small portion (less than 25 per cent) of the deflation hyperemia can be due to the filling of vascular segments whose pressure is temporarily lowered during the period of decompression. The remainder of the deflation hyperemia differs strikingly from reactive hyperemia in its sensitivity to cocaine, and in its relation to the duration of the inciting cause. Its mechanism is therefore probably not identical with that of reactive hyperemia.

The assignment of a minor rôle to these two mechanisms makes it probable that the greater part of the deflation hyperemia is due to persistence of a resistance-lowering mechanism set up by stretch of the intestinal walls.

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PHENYLTHIOCARBAMIDE TASTE THRESHOLDS OF RATS AND HUMAN BEINGS

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Previous papers contain a description of a method for the determination of taste thresholds which can be used both for rats and human beings. With this method the taste thresholds have already been determined for the two most common substances which appear in our diet in purified form, common salt and sugar. Rats showed that they could first distinguish a sodium chloride solution from distilled water when the concentration reached 0.055 gram per cent (1); human beings first recognized a difference between sodium chloride solution and distilled water in concentrations of 0.016 per cent and first recognized a "salt" taste in concentrations of 0.087 per cent (2). Rats first showed that they could distinguish a sucrose solution from distilled water in concentrations of 0.5 per cent; human beings first recognized a difference between sucrose solution and distilled water in concentrations of 0.17 per cent and first recognized a "sweet" taste in concentrations of 0.41 per cent (3). Thus, for these two common substances human beings and rats have very nearly the same taste thresholds.

A further study has now been undertaken on the taste thresholds of a highly bitter tasting substance, phenylthiocarbamide. This substance was used partly because it has already been employed in numerous studies on taste thresholds (4, 5) and on the inheritance of taste ability (6, 7, 8, 9, 10, 11) and partly because, unlike sodium chloride and sucrose, it has a highly toxic effect on rats, 1 to 2 mgm. being sufficient to kill them in only a few hours (12).

METHODS. The rats were kept separately in cages, each of which contained a food cup and two graduated inverted 100 cc. bottles. The same two bottles were used in each cage throughout the experiment; and each bottle was marked and kept in the same corner of the cage, either on the right or on the left side. The fluid intake from the two bottles was recorded daily. For approximately 10 days both bottles were filled each day with distilled water. At the end of this time the intake from each bottle usually became fairly constant. Then one bottle was filled with a

subliminal concentration of a solution of phenylthiocarbamide, made with distilled water; and each day thereafter the concentration of the solution was increased in small steps. As the concentrations were increased, a point was finally reached at which the rats began to drink less phenylthiocarbamide solution and more water. The concentration at which they thus first indicated that they recognized the difference between phenylthiocarbamide solution and distilled water was taken as the taste threshold. The temperature of the solution varied with the room temperature which ranged from 24° to 26°C.

Of the 47 rats, 23 were males and 24 females. Records were kept of litter mates. The ages of the rats ranged from 70 to 152 days.

In the threshold determinations on human beings the subjects were seated at a table opposite the experimenter. Two one-ounce glasses, each containing about 5 cc. of distilled water, were placed in the subject's hands with instructions to sample the fluids and to describe the taste of each one. Then the two glasses were presented again, one containing distilled water, the other a subliminal concentration of phenylthiocarbamide. With each successive trial the concentration was increased in the same steps used for the rats. One glass always contained distilled water, the other a solution of phenylthiocarbamide. The glasses were indistinguishable, except for a small mark which was visible only to the experimenter. Their relative positions, to the right or to the left, were varied in an irregular order. A record was kept of the sensations described by the subjects for each concentration. When the subjects definitely stated that phenylthiocarbamide had a bitter taste or when it was found that they could not taste a 0.25 per cent solution, the test was discontinued. To make certain that no difference in temperature existed between them, the phenylthiocarbamide solution and the distilled water were checked at frequent intervals. They were kept at room temperature which ranged from 22° to 26°C.

Of the 261 human subjects, 139 were males and 122 were females. The ages varied from 17 to 50 years. Most of the subjects were students at the State Teachers College at Towson, Maryland, and at Loyola College; the others were members of the laboratory and clinical staffs at the Phipps Psychiatric Clinic.

Results. *Taste thresholds of rats.* Figure 1 shows the record of a typical rat. The ordinates give fluid intake in cubic centimeters; the abscissas, time in days and concentrations of phenylthiocarbamide. During the preliminary period of seven days on two bottles of distilled water, the rat drank more from bottle 1 than from bottle 2. On the eighth day phenylthiocarbamide was added to bottle 1, from which the rat had been drinking more water. After 14 days, when the concentration of phenylthiocarbamide had reached 0.0003 per cent, the animal first began to drink less of the phenylthiocarbamide solution. This was taken as the taste

threshold. The rat completely refused all higher concentrations and drank only from the water bottle.

Figure 2A gives the frequency curves of taste thresholds for the 47 rats. The taste thresholds ranged from 0.00005 to 0.02 per cent. The maximum frequency fell at 0.0003 per cent. Two rats did not begin to drink less of the solution until the concentration reached 0.02 per cent.

Taste difference thresholds of human beings. Figure 2B shows the concentrations of phenylthiocarbamide solution at which each of the 261 human beings first stated that they detected a taste different from that of distilled water. They ranged from 0.000005 to 0.1 per cent. The maximum frequency fell at 0.0003 per cent, which is also the taste threshold for rats.

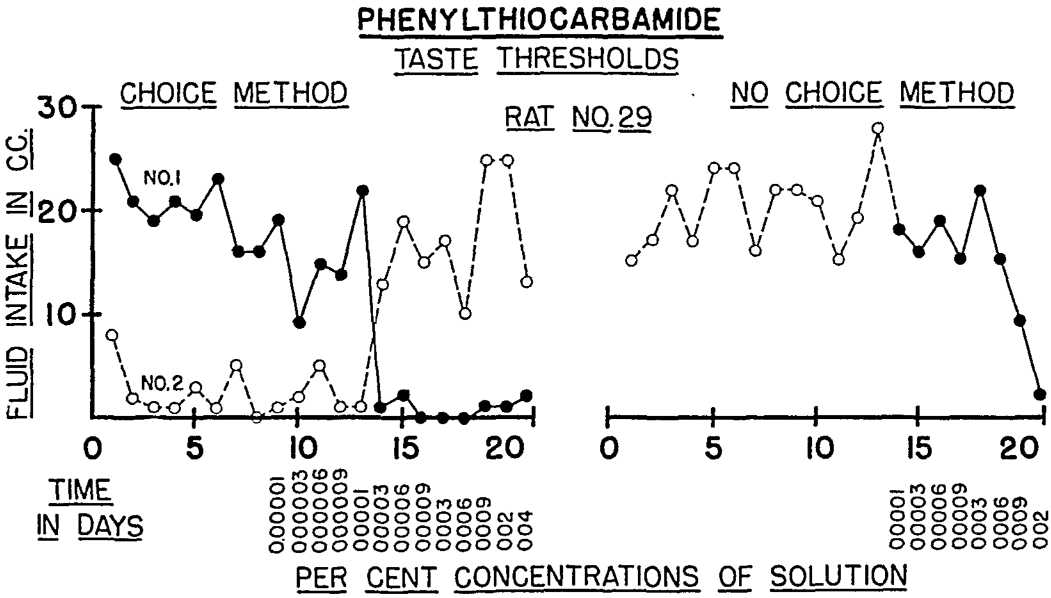

Fig. 1

When they first recognized a difference between the phenylthiocarbamide solution and distilled water, 125 of the 261 human subjects stated that it had a bitter taste; 136 subjects recognized a difference but did not report a bitter taste. Table 1 gives a list of the different taste expressions and the frequency of their use by these individuals.

Bitter taste thresholds of human beings. The concentrations of the phenylthiocarbamide solution at which the 261 human subjects first recognized a bitter taste ranged from 0.00001 to 0.2 per cent. See figure 2C. The maximum frequency, however, fell at 0.0003 per cent, which is the same concentration found for the difference thresholds for the same human subjects and for the taste thresholds of rats. At water saturation of phenylthiocarbamide (0.25 per cent) 13 individuals failed to recognize a bitter taste. These same individuals had, however, recognized a differ-

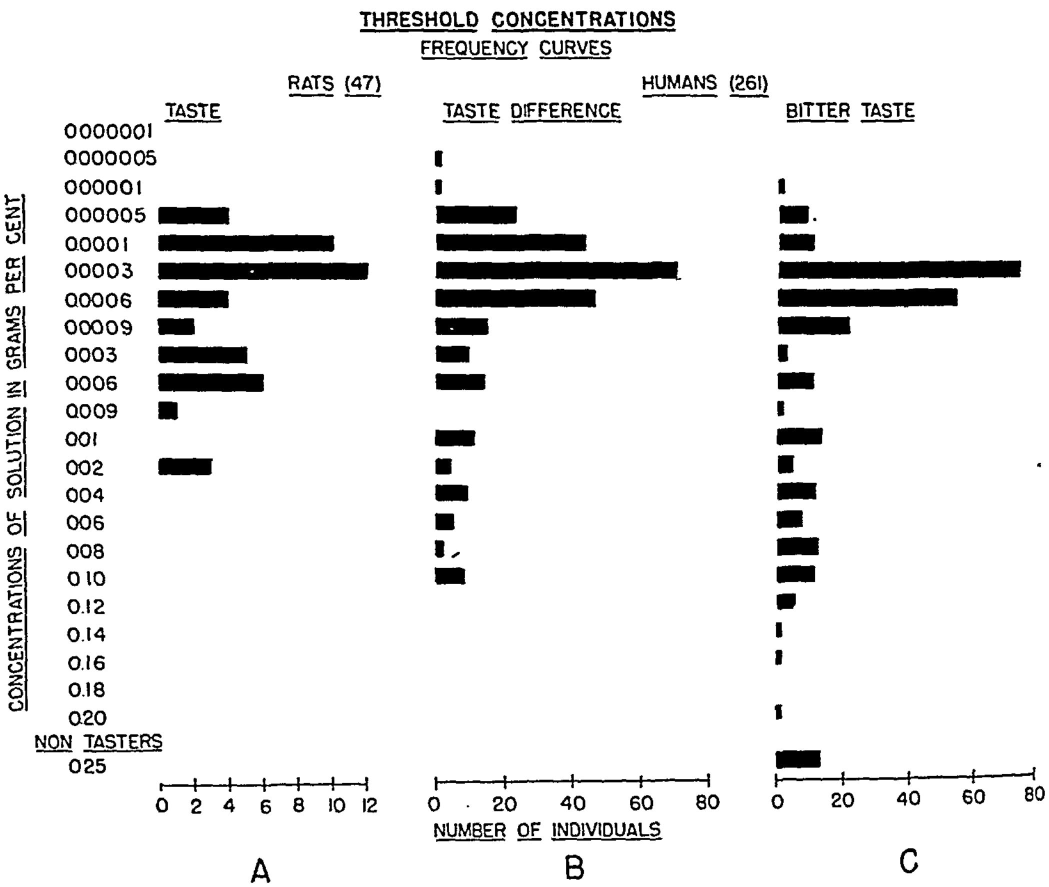

Fig. 2

TABLE 1

Expressions used to describe subliminal phenylthiocarbamide solutions as compared with distilled water and frequency of use

TASTE SENSATIONS	NUMBER OF SUBJECTS
Different	33
Stronger	18
Dry	11
Alum—Puckering	5
Burning	2
Sweet	15
Salty	5
Sour	17
Iron	2
Other sensations and combinations of the above	28
Total	136

ence in taste between the phenylthiocarbamide solution and distilled water. They objected to the taste of the phenylthiocarbamide even though it did not taste bitter to them. These results confirm the work of Salmon and

Blakeslee (5), who report a maximum frequency for bitter responses at 0.000312 per cent. In their study on 93 individuals, 3 per cent were non-tasters.

REMARKS. With the hope of simplifying the technique used on rats we attempted to determine the taste threshold with one bottle in place of two. For about 10 days this bottle was filled with distilled water, and daily records were taken to obtain a base line of fluid intake. Then the bottle was filled with a subliminal concentration of phenylthiocarbamide solution, and the concentrations were increased each day exactly as with the two-bottle technique. With this method the maximum frequency fell at 0.009 per cent, which was considerably above the threshold of 0.0003 per cent obtained with the two-bottle technique. The concentrations from 0.003 to 0.009 per cent included 41 of the 47 rats. At these concentrations the distastefulness of the solution must have overcome the thirst. Clearly, the taste threshold given by this method is far above the actual threshold. Figure 1 shows a typical record obtained with the one-bottle or no choice method.

Taste as a guide. The results of these experiments have demonstrated that most of the rats avoided the highly toxic phenylthiocarbamide even in very low concentrations. The question arises, then, as to whether rats could be made to take phenylthiocarbamide in lethal doses if it were offered in a solution with some substance for which they have a strong craving, or simply if it were mixed with the regular stock diet.

In one set of experiments 8 rats kept on the stock diet were offered a choice between plain water and a 20 per cent dextrose solution which contained 0.02 per cent of phenylthiocarbamide. Table 2 summarizes the results. For the 10 days before the phenylthiocarbamide was added the daily intake of the 20 per cent dextrose solution averaged 62 cc. When phenylthiocarbamide was added to the dextrose solution, the daily intake dropped sharply to 6.6 cc. for the first 24 hours. From their intake of this solution the rats received from 0.8 to 2.0 mgm. of phenylthiocarbamide per day—that is, amounts which fall at the lower limits of the lethal dose. Four of the rats died within 12 hours; the other 4 apparently did not drink enough of the dextrose solution to kill them. They gave no indications of any toxic effects.

This experiment was repeated on 8 rats with twice the amount of phenylthiocarbamide added to the dextrose solution (0.04 instead of 0.02 per cent). Here again 4 rats died and 4 showed no signs of toxic effects. The daily phenylthiocarbamide intake of the rats which lived ranged from 0.04 to 2.0 mgm. and of those that died, from 1.6 to 2.8 mgm. The determining factor in such an experiment would presumably be the amount of phenylthiocarbamide ingested at any one time.

In another experiment we offered 9 rats a choice between the regular

stock diet in one cup and the stock diet mixed with a certain amount of phenylthiocarbamide in a second cup. For 3 rats the food contained 500 mgm. of phenylthiocarbamide per 100 grams, or 0.5 per cent; for 3 rats the food contained 200 mgm. of phenylthiocarbamide per 100 grams, or 0.2 per cent; and for 3 rats it contained 100 mgm., or 0.1 per cent. Table 3 summarizes the results. The first 3 rats took from 2 to 3 grams of the phenylthiocarbamide food, thus ingesting 10 to 15 mgm. of phenylthio-

TABLE 2

RAT NUMBER	SEX	INTAKE OF DEXTROSE, 20 PER CENT, 10-DAY AVERAGE	INTAKE OF PTC, 0.02 PER CENT, AND DEXTROSE, 20 PER CENT, 1 DAY	INTAKE OF PTC	SURVIVAL TIME
		cc.	cc.	mgm.	
1	♂	62	10.0	2.0	12 hours or less
2	♂	70	8.0	1.6	12 hours—died
3	♂	44	6.0	1.2	12 hours—died
4	♂	74	6.0	1.2	12 hours—died
5	♂	59	9.0	1.8	Survived
6	♂	56	5.0	1.0	Survived
7	♂	60	5.0	1.0	Survived
8	♂	67	5.0	0.8	Survived
Average		62	6.6		
			PTC, 0.04 PER CENT, AND DEXTROSE, 20 PER CENT		
9	♂	65	7.0	2.8	12 hours—died
10	♂	60	5.0	2.0	12 hours—died
11	♂	53	4.0	1.6	12 hours—died
12	♂	53	4.0	1.6	12 hours—died
13	♂	65	5.0	2.0	Survived
14	♂	59	4.0	1.6	Survived
15	♂	49	2.0	0.8	Survived
16	♂	60	1.0	0.4	Survived
Average		57	4.5		

carbamide. They all died within a few hours. The second 3 rats ingested 1 to 2 grams of the phenylthiocarbamide food, thus getting 2 to 4 mgm. of phenylthiocarbamide. All 3 died. One of the last 3 rats ate 2 grams of the phenylthiocarbamide food and died; one rat ate 1 gram; the other did not eat a sufficient amount of food for weight determination. The latter 2 rats survived.

The high mortality of the rats that received the phenylthiocarbamide in their food may be explained by the high degree of insolubility of the

phenylthiocarbamide in powdered form. Undoubtedly the rats sampled the food in both cups. Since the phenylthiocarbamide is so highly insoluble, they must have eaten a lethal dose of the phenylthiocarbamide food before they recognized the bitter taste. The 50 per cent mortality of the rats which received phenylthiocarbamide in the dextrose solution may be explained partly by the possibility that the sweetness of the dextrose may have greatly reduced the bitter taste of the phenylthiocarbamide and partly by the possibility that the craving for the dextrose overcame the aversion to the phenylthiocarbamide. Since taste thresholds were not determined previously on these rats, it is possible that the animals that died may have been less sensitive to the bitter taste.

TABLE 3

RAT NUMBER	SEX	INTAKE		PTC IN FOOD	INTAKE OF PTC	SURVIVAL TIME
		Regular food	PTC food			
		grams	*grams*	*per cent*	*mgm.*	
17	♀	0	3	0.5	15	12 hours or less
18	♀	2	2	0.5	10	12 hours—died
19	♀	2	2	0.5	10	12 hours—died
20	♀	1	2	0.2	4	12 hours—died
21	♀	0	2	0.2	4	12 hours—died
22	♀	2	1	0.2	2	12 hours—died
23	♀	2	2	0.1	2	12 hours—died
24	♀	2	1	0.1	1	Survived
25	♀	2	0	0.1	0	Survived

Discussion. The results of these experiments bring further evidence for the close gustatory relationship of rats and human beings. We know now that rats and human beings have almost identically the same taste thresholds for common sugar, salt, and phenylthiocarbamide.

Since about 5 per cent of human beings scarcely taste the substance, or do not get its bitter taste at all, they very likely would take dangerously large amounts, even if the phenylthiocarbamide were offered in an aqueous solution. Human beings who are taste blind to phenylthiocarbamide and similar substances should be helped in their dietary selections and avoidances just as color blind individuals are helped by special signs and warnings.

SUMMARY

1. For the 47 rats the taste threshold concentrations ranged from 0.00005 to 0.02 per cent. The maximum frequency fell at 0.0003 per cent.

2. For the 261 human subjects the concentrations at which the phenylthiocarbamide solution first tasted different from distilled water ranged from 0.000005 to 0.1 per cent. The maximum frequency fell at 0.0003 per cent, at which concentration rats first indicated that they recognized a difference between the phenylthiocarbamide solution and distilled water.

3. The concentrations at which human subjects first recognized a bitter taste ranged from 0.00001 to 0.2 per cent. The maximum frequency for the 261 individuals again fell at 0.0003 per cent. Thirteen individuals never recognized a bitter taste even though they did recognize that the phenylthiocarbamide did not have the same taste as the distilled water. These results confirm those of Salmon and Blakeslee (5).

4. The concentration at which 95.5 per cent of the rats refused to take the phenylthiocarbamide solution fell below the lethal doses.

5. It was found that when phenylthiocarbamide was mixed with regular food most of the rats would eat enough to kill themselves. This may be explained by the fact that, due to the insolubility of phenylthiocarbamide, they swallow it before they taste it. When the phenylthiocarbamide was placed in a 20 per cent dextrose solution, 50 per cent of the rats drank sufficiently large amounts to kill themselves. The sweet flavor of the dextrose may have concealed the bitter taste of the phenylthiocarbamide.

These experiments were greatly aided by the coöperation of Rev. R. B. Schmitt, S. J., of Loyola College and Dr. M. Theresa Wiedefeld of the State Teachers College at Towson, Maryland.

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BLOOD VOLUME CHANGES IN MEN EXPOSED TO HOT ENVIRONMENTAL CONDITIONS FOR A FEW HOURS[1]

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Studies of blood volume changes occurring in various environments made by the use of indirect methods (1, 2, 3) have yielded somewhat conflicting results. With the carbon monoxide and dye methods (4, 5, 6) increases in blood volume have been noted in subjects exposed to warm environments for appreciable lengths of time. Decreases occurred when they were returned to cool surroundings. Other workers have found either small (7) or no changes (8, 9) in blood volumes of subjects after acclimatization to warm environments. The authors (10) have reported increases in plasma volume of subjects exposed to hot environments for 2 to 3 hours. The control experiments, under comfortable conditions, were done 7 to 10 days prior to the day of the experiment. This work was open to the criticism that the physiological states of the individuals might have been altered in the interval. It seemed wise to repeat the experiments measuring the control blood volume on the day of the experiment.

Experimental procedures. The plan called for two blood volume determinations to be carried out on the same subject the same morning; the first in a comfortable environment; the second after exposure to heat.

Subjects. The subjects were all healthy male medical students ranging from 20 to 27 years of age. They had slept in the room the night before under comfortable conditions. Prior to entrance they drank freely of water. All determinations were carried out on the nude subjects under basal conditions. When the subject was weighed this was done immediately after each blood volume was completed. As a precaution (11, 12)

[1] This work was made possible by a grant of the American Society of Heating and Ventilating Engineers.

the subject was again recumbent for at least 90 minutes before the second blood volume was started. Correction was made for the blood withdrawn.

The room. Conditions in the experimental room were first 28.6°C. dry bulb and 19.7°C. wet bulb. This constituted the "comfortable environment" which is in the "neutral zone" of DuBois (13). Air movement was constant and minimal, and the temperature of the walls was almost identical with the temperature of the air. After the first blood volume determination, the thermostat and humidistat were changed to the desired "hot" readings. Usually about 45 to 55 minutes were needed for the room to warm up. At this time the surface temperature of the globe thermometer was within 0.4°C. of the air temperature.

The temperatures of the hot environments were varied but had in common a dry bulb as high as the normal body temperature, or higher. This compelled the heat loss to be entirely evaporative.

OBSERVATIONS. The blood volumes were determined by the method of Gibson and Evelyn (14) using the dye T1824 and the Cenco-Sheard photelometer. Additional observations, which included blood counts, blood protein analyses, rectal temperature, and vital capacity were all made in the comfortable and again in the hot conditions.

RESULTS. *Controls.* Table 1 shows the results in 5 subjects on whom blood volume determinations were carried out twice in the same morning, the conditions being identical. These experiments show excellent agreement.

Plasma volume increased. Table 2 shows those instances in which an increase in the circulating plasma volume of 5 per cent or more was observed. One notices that the extent of increase in circulating plasma volume may be considerable, reaching 13 per cent which is more than four times the maximum variability observed in the control series. The volume of red cells in circulation also increased appreciably. These increases occurred despite the loss of significant quantities of body water. The wet bulb was rather low in all but one experiment (27.5°C.). In an atmosphere at this wet bulb and a dry bulb of 37.3° to 37.8° gross sweating will appear. Hence the environments used were those conducive to a ready evaporative loss of heat. The heat produced was almost completely lost as indicated by the slight changes in rectal temperature. Apparently *mobilization of fluids is an early adjustment for heat elimination.* The fluids consist of blood which is presumably stored in the so-called "blood depots," the capillaries of the spleen, liver, lungs, and probably muscles.

Plasma volume decreased. In four experiments a diminution in circulating plasma volume of more than 5 per cent was observed (table 3). It is easy to believe that in the last two experiments on this table, the subjects were in a relative state of anhydremia resulting from exposure of more than three and one-half hours to very hot (44.7°C. dry bulb) conditions with a

TABLE 1

Controls

SUBJECT AND DATE	MINUTES BETWEEN DYE INJECTIONS	PLASMA VOLUME	RBC VOLUME
		cc.	*cc.*
M. A.	0	3497	2896
12/10/39	140	3498	2839
J. W.	0	2976	2747
12/20/39	149	2966	2679
F. R.	0	2083	1699
1/2/40	153	2042	1691
C. N.	0	3226	2453
1/8/40	138	3179	2370
P. H.	0	2941	1403
1/9/40	129	2861	1473

TABLE 2

Plasma volume increased

SUBJECT AND DATE	CONDITIONS		EXPOSURE TIME	PLASMA VOLUME	PER CENT CHANGE	RBC VOL.	PER CENT CHANGE	RECTAL TEMP.	WEIGHT LOSS
	Dry bulb	Wet bulb							
	°C.	*°C.*	*minutes*	*cc.*		*cc.*		*°C.*	*grams*
C. G.	Comf.*			3049		2485		36.8	
6/3	37.2	22.1	130	3448	+13.1	2731	+9.9	37.4	
A. M.	Comf.			2583		2349		37.2	
9/23	37.6	27.5	59	2866	+10.9	2742	+16.7	37.2	381
F. C.	Comf.			2840		2232		36.6	
9/5	37.3	24.2	162	3122	+9.9	2589	+16.0	36.9	596
H. C.	Comf.			3367		2468		36.9	
6/7	37.2	24.3	138	3663	+8.8	2855	+15.7	37.4	
F. C.	Comf.			3380		2575		36.3	
7/26	44.7	26.4	216	3572	+5.7	2832	+10.0	36.9	
H. C.	Comf.			3012		2802		37.0	
5/28	37.2	20.1	160	3165	+5.4	2924	+4.4	37.5	

* Comf. = Dry bulb 28.6, wet bulb 19.7.

wet bulb reading of 27.1° and 27.5°C. However, the fall in circulating plasma volume is rather low, less than the increases reported above. The

instance of R. G. on 8/30, when comparatively short exposure of 156 minutes to a moderately hot environment (37.3°C. dry bulb, 20.3°C. wet bulb) led to a fall in blood volume without a rise in rectal temperature of over 37.0°C., is difficult to understand. Further studies will be required to understand the conditions under which plasma concentration occurs following relatively short exposures to hot environments.

Plasma volume unchanged. Table 4 shows results in many cases having in common the observation of less than 5 per cent change in circulating plasma volume. The chief fact obvious at a glance is that the extent of change in plasma volume is not related to the quantity of water lost by sweating. One man lost 1104 grams in weight while showing a change

TABLE 3

Plasma volume decreased

SUBJECT AND DATE	CONDITIONS		EXPOSURE TIME	PLASMA VOLUME	PER CENT CHANGE	RBC VOL.	PER CENT CHANGE	RECTAL TEMP.	WEIGHT LOSS
	Dry bulb	Wet bulb							
	°C.	*°C.*	*minutes*	*cc.*		*cc.*		*°C.*	*grams*
S. J.	Comf.			3164		2137		36.4	
9/27	37.5	29.7	69	2977	−5.9	2042	−4.4	37.2	450
R. G.	Comf.			3196		2583		36.6	
8/30	37.2	20.3	156	2995	−6.3	2463	−4.6	36.9	546
R. G.	Comf.			3547		2732		36.7	
7/24	44.7	27.1	216	3336	−6.0	2983	+9.2	37.6	
A. M.	Comf.			3333		2876		36.9	
7/22	44.6	27.5	226	3119	−6.4	2960	+2.9	38.1	

of about the same magnitude in circulating plasma volume as that shown by another subject after losing only 569 grams.

Apparently, the experiments detailed in this table show that the two physiological processes, which produce opposite effects on the blood volume, may be so combined as to neutralize each other. Thus the expansion of the volume by the flow of blood from the reservoirs goes on simultaneously with the evaporation of water from the plasma spread out in the capillaries. By selecting conditions one or the other process may predominate and give an increase or decrease in volume. More commonly no change is noted.

As the exposure to the hot environment continues, new supplies of water are requisitioned from the tissues to replace that evaporated from the plasma. The quantity of water so transferred is indicated as weight loss in grams. Once the blood volume has been increased with the addition

TABLE 4

Plasma volume unchanged

SUBJECT AND DATE	CONDITIONS		EXPOSURE TIME	PLASMA VOLUME	PER CENT CHANGE	RBC. VOL.	PER CENT CHANGE	RECTAL TEMP.	WEIGHT LOSS
	Dry bulb	Wet bulb							
	°C.	°C.	*minutes*	*cc.*		*cc.*		°C.	*grams*
S. J.	Comf.			3105		2251		36.7	
9/11	37.8	18.7	169	3140	+1.1	2372	+5.4	37.2	652
R. G.	Comf.			3817		3249		36.8	
8/23	37.4	20.6	160	3725	−2.4	3162	−2.7	37.0	569
J. W.	Comf.			3497		2815		36.9	
5/31	38.0	20.6	91	3546	+1.4	2820	+0.2	37.3	
T. S.	Comf.			3690		3094		36.4	
9/3	37.5	22.4	161	3821	+3.6	3322	+7.4	37.1	554
J. W.	Comf.			3322		2606		36.7	
6/10	37.2	22.7	148	3333	+0.3	2760	+5.9	37.1	
A. M.	Comf.			3185		2859		36.8	
8/14	37.6	25.6	186	3236	+1.6	2951	+3.2	37.4	816
T. S.	Comf.			4255		3044		36.5	
10/1	37.5	27.0	60	4429	+4.1	3217	+5.7	37.1	335
W. H.	Comf.			2398		2484		36.8	
9/19	37.6	28.1	132	2426	+1.2	2632	+6.0	37.3	652
R. G.	Comf.			3164		2601		36.6	
7/16	37.3	28.3	205	3259	+2.9	2822	+8.5	37.2	
A. M.	Comf.			3108		2757		37.0	
8/8	37.6	28.6	201	3114	+0.2	2877	+4.4	37.8	
C. G.	Comf.			3303		2497		36.8	
6/21	37.3	29.7	182	3354	+1.5	2613	+4.6	37.6	
H. C.	Comf.			3509		2930		36.9	
6/24	37.8	30.1	201	3496	−0.4	3075	+5.0	37.9	
T. S.	Comf.			4423		3169		36.7	
10/23	44.2	21.6	187	4277	−3.3	3241	+2.3	37.4	1104
S. J.	Comf.			3154		2383		36.7	
10/25	44.3	23.5	188	3039	−3.6	2320	−2.6	37.6	1279

of new blood from the body reservoirs, the cells remain even though the plasma may be subsequently reduced by evaporation. This is shown by a

significant increase in the circulating volume of red cells in 10 out of 14 experiments in the plasma unchanged group.

Plasma volume increased, unchanged, and decreased in two subjects. In two subjects (table 5) the plasma volume was found to show an increase, no change, and a decrease. The increases occurred when the subjects had been exposed through relatively short periods. The decreases were noted after longer exposures to more severe conditions.

TABLE 5

Plasma volume increased, unchanged and decreased in two subjects

SUBJECT AND DATE	CONDITIONS		EXPOSURE TIME	PLASMA VOLUME CHANGE	PER CENT CHANGE	RBC VOL. CHANGE	PER CENT CHANGE	PER CENT PROTEIN	TOTAL CIRCULATING SERUM PROTEIN	RECTAL TEMP.	WEIGHT LOSS
	Dry bulb	Wet bulb									
	°C.	°C.	minutes	cc.		cc.				°C.	grams
A. M.	Comf.			2583		2349		6.68	173	37.2	
9/23	37.6	27.5	59	2866	+10	2742	+16.7	6.72	193	37.2	381
8/8	Comf.			3279		2757		6.95	228	37.0	
	37.6	28.6	201	3216	−1.9	2877	+4.4	6.57	211	37.8	
7/22	Comf.			3333		2876		6.66	222	36.9	
	44.6	27.5	226	3119	−6.4	2960	+2.9	7.23	225	38.1	
T. S.	Comf.			4255		3044		6.78	289	36.5	
10/1	37.5	27.0	60	4429	+4.0	3217	+5.7	6.69	296	37.1	335
9/3	Comf.			3690		3094		7.06	261	36.4	
	37.5	22.4	161	3821	+3.3	3322	+7.4	6.88	263	37.1	554
2/9	Comf.			4000		3220				36.3	
	37.7	30.7	150	3968	−0.3	3173	−1.4			37.6	488
10/23	Comf.			4423		3169				36.7	
	44.2	21.6	187	4277	−3.3	3241	+2.3			37.4	1104

Blood counts. Counts of red and white blood cells were done in duplicate at approximately the same time the dye was injected for the blood volume determinations The blood was obtained by puncturing the ear lobe. The dilutions and counts were made by the same individual throughout (M. M.). Care was taken to obtain thorough mixing of the cells with the diluting fluid and the pipettes were always rotated immediately prior to depositing the suspension in the counting chamber.

It is apparent that there are no significant changes in red cell count except for the two cases having a relative anhydremia. There are significant alterations in white cell count in 10 out of 24 experiments. Since

[illegible] it is difficult at this time to attribute any significance to them.

Serum proteins. Total serum protein was determined by Van Slyke's [illegible] micro-Kjeldahl (15) method. The albumin was separated from the globulin by Howe's method (16). In every case in which there was an increase in plasma volume (table 6) and in which serum proteins were determined [illegible] per cent change was observed, with one exception (H. C. 6/7). The total circulating plasma proteins expressed in grams showed an increase in all cases except one (H. C. 6/7). This result is in accord with those reported by Barbour, Loomis et al. (3) and

TABLE 6

Plasma volume increased

Subject and date	[illegible]	[illegible]	Hb	Serum proteins: Total	Alb.	Glob.	Total circulating plasma protein	[illegible]
	[illegible]						grams	
C. [illegible]	5.94	5.90	16.3					44.9
[illegible]	5.[illegible]2	6.27	15.1					44.3
A. M.	5.25	7.[illegible]	16.[illegible]	6.68	4.5[illegible]	2.12	173	47.6
9/2[illegible]	5.1[illegible]	7.15	16.6	6.72	4.61	2.11	19[illegible]	48.9
[illegible]	5.84	7.75	15.8	6.22	4.65	1.57	177	44.0
6/2	5.62	6.80	15.7	6.38	4.60	1.78	19[illegible]	46.3
H. C.	6.17	8.05	12.[illegible]	6.19	4.66	1.53	208	42.3
6/7	6.08	7.65	11.[illegible]	5.56	4.30	1.26	204	43.8
[illegible]	5.21	6.85	15.0	6.11	4.26	1.88	218	43.2
7/2[illegible]	5.35	8.20	15.2	6.24	4.49	1.75	223	44.2
H. C.	6.42	4.8[illegible]	16.7	6.11	4.70	1.41	18[illegible]	48.2
5/2[illegible]	6.87	6.75	16.2	6.58	4.30	1.98	202	48.0

Bazett et al. (6). Evidently, the increased plasma volume is composed of fluid having about the same protein concentration as the normal plasma.

The same situation exists in the unchanged group (table 8) where just one subject (A. M. 8/8) shows a drop of questionable significance comprising 5.5 per cent of the initial protein concentration. The grams of total circulating plasma proteins also were unchanged. In the concentration group (table 7) there was a percentage increase in the plasma proteins in two cases without change in the total circulating proteins (expressed in grams). This again indicates that the concentration is attributable to the evaporation of water and should be considered as an anhydremic phe-

nomenon. The results on R. G. 8/30 are not in agreement and at present are not explained.

Changes in pulse rate. The correlation between the pulse rate and the rectal temperatures of subjects in a steady state exposed to hot environments of varying degrees has been previously reported (17). From 101 observations on 5 subjects it was noted that an average increase in pulse rate per degree centigrade rise in rectal temperature was 25 beats. At that time changes in plasma volume were not considered. In table 9 the correlation is now extended to include both plasma volume changes and changes in rectal temperatures. It is evident that an increase in plasma volume tends to minimize the increase in pulse rate. The primary correlation therefore, of pulse rate increase, is with a rise in rectal temperature and the secondary one with plasma volume changes.

TABLE 7

Plasma volume decreased

SUBJECT AND DATE	R.B.C.	W.B.C.	Hb.	PER CENT PROTEINS			TOTAL CIRCULATING SERUM PROTEINS	HEMATOCRIT
				Total	Alb.	Glob.		
	millions	*thousands*					*grams*	
S. J.	4.72	8.57	13.5	6.12	4.60	1.52	194	40.3
9/27	4.39	9.50	13.7	6.28	4.55	1.73	187	40.7
R. G.	4.70	7.90	15.2	6.53	4.86	1.67	209	44.7
8/30	5.00	9.55	15.3	6.00	4.97	1.03	180	45.1
R. G.	5.45	7.35	15.3	6.54	4.80	1.74	232	43.5
7/24	6.23	7.00	16.2	7.29	5.18	2.11	243	47.2
A. M.	5.35	7.38	16.2	6.66	4.81	1.85	222	46.3
7/22	6.11	6.10	17.3	7.23	5.22	2.01	225	48.8

DISCUSSION. The difficulties in the measurements of plasma volume are many. Although these have been reduced by the studies of Rowntree (18), Gregersen (19), Gibson (8) and others (20, 21), one still encounters pitfalls. It can be readily seen that deductions as to plasma volume changes derived by following hemoglobin, specific gravity, total solids, and hematocrit determinations are unreliable. Thus in the experiments in which the plasma showed either a dilution or concentration, there were no significant changes in these values.

With the dye method as now standardized, one would expect to secure over a period of time reproducible plasma volumes in any given subject. Reference to table 5 shows that the plasma volumes of two subjects who should have been in a steady state under our "comfortable environment" were not the same from day to day. Similar discrepancies have been

TABLE 8

Plasma volume unchanged

SUBJECT AND DATE	R.B.C.'S	W.B.C.'S	Hb.	PER CENT PROTEIN			TOTAL CIRCULATING SERUM PROTEINS	HEMATOCRIT
				Total	Alb.	Glob.		
	millions	*thousands*					*grams*	
S. J.	4.72	10.05	14.7	6.42	4.63	1.79	199	42.0
9/11	4.78	7.85		6.68	4.47	2.21	208	43.0
R. G.	5.27	7.70	15.9					46.0
8/23	5.30	7.60	15.9					45.9
J. W.	5.01	7.80						44.6
5/31	5.15	4.50						44.3
T. S.	4.85	5.47		7.06			260	45.7
9/3	4.77	5.40		6.88			263	46.5
J. W.	5.08	7.25	14.8					44.0
6/10	5.22	5.60	15.4					45.3
A. M.	5.38	6.92	16.6	6.76	4.90	1.86	215	47.3
8/14	5.35	6.15	16.4	7.08	5.04	2.04	229	47.7
T. S.	4.69	5.25	14.8	6.78	4.65	2.13	288	41.7
10/1	4.62	4.72	14.9	6.69	4.56	2.13	296	42.1
W. H.	5.27	9.22	17.7					50.9
9/19	5.68	9.10	17.8					52.0
R. G.	5.17	8.00	15.8	6.71	4.72	1.99	212	45.1
7/16	5.27	8.25	16.2	6.72	4.84	1.88	219	46.5
A. M.	5.60	9.47	17.0	6.95	4.73	2.22	216	47.0
8/8	5.35	9.45	16.9	6.57	4.78	1.79	204	48.0
C. G.	5.03	7.12	14.8	6.65	4.57	2.08	220	43.1
6/21	5.32	5.72	15.3	6.84	4.75	2.09	229	43.8
H. C.	5.67	7.45	15.3	6.60	4.66	1.94	231	45.6
6/24	5.74	7.22	15.8	6.79	5.10	1.69	237	46.8
T. S.	4.73	5.20	14.8					41.7
10/23	4.66	6.22	15.3					43.1
S. J.	4.74	9.37						43.0
10/25	4.59	9.70						43.3

found in the literature. This is not necessarily a reflection on the method or the technique of its application. Apparently the circulating plasma

volume in any individual is not necessarily fixed. It is not always the same day after day. There is a constant phasic adjustment between the circulating blood volume and the vascular bed. Patients with large spleens (22) have large blood volumes. After the spleen is removed the volume is decreased. The plasma volume drops (10, 11) when the individual assumes the standing position. When the atmospheric environment is cool the quantity of blood in the lungs as measured by the vital capacity is larger than when the environment is hot (23). When the surroundings are warm the blood shifts to the periphery of the body.

It now becomes apparent that one must exercise extreme caution in interpreting blood volume estimations from day to day, such as have been reported in acclimatization studies. Consequently, we believe our procedure of determining the blood volume immediately before and after an experiment is a more reliable index of a given physiological adjustment. It would seem that in the adjustment to hot environments the plasma volume regularly increases. In a number of our experiments this was

TABLE 9

Pulse rate changes

PLASMA VOLUME	EXPERIMENTS	AVERAGE INCREASE OF PULSE/°C. RISE IN RECTAL TEMPERATURE
Increased	6	7
Unchanged	14	18
Decreased	4	34
Not determined	101	25

found to be between 5 per cent and 13 per cent. In others it was not observed but an increase in red cell volume was noted. In these latter experiments it would seem that water was lost from the plasma, but the cells which had been swept into the circulation during the dilution phase remained behind and were observed as an increased red cell volume. It was further noted that in those subjects whose plasma volume increased there was no significant rise in the pulse as the rectal temperature rose. This suggests that some subjects have a readily mobilizable reserve plasma volume. With the rise in temperature the peripheral vessels dilate and the increased vascular bed is filled with this reserve plasma. Other subjects who are unable to supply adequate plasma, or whose plasma volume is reduced by evaporation, develop a disparity between the blood volume and the vascular bed. An example of this acute disparity was previously reported by the authors (17). The inability of certain individuals to withstand heat may be attributed to the disparity which arises when the plasma volume fails to increase on exposure to hot conditions.

CONCLUSIONS

1. Subjects were exposed to environments having a dry bulb of 37.2 and 44.7 degrees C. and wet bulb temperatures of 20.1 to 27.5 degrees. The globe thermometer showed less than 0.4°C. difference between its surface and the surrounding air. Air currents were minimal. The periods of exposure ranged from 59 to 160 minutes. In 6 experiments there was an increase in circulating plasma volume, red cell mass, and grams of total circulating serum proteins, all of which would be expected on the assumption that the new fluids were contributed by blood from the body reservoirs (spleen, and inactive capillary beds in muscles, lungs, and viscera).

2. In 4 other experiments in which the dry and wet bulbs showed approximately the same ranges, there was a decrease in the circulating blood plasma and variable changes in the red cell mass. The periods of exposure ranged from 69 to 226 minutes. The changes in the plasma proteins, red and white cell counts were such as would be explained on the assumption that water was lost by evaporation from the blood plasma.

3. In 14 experiments on subjects exposed to the same type of environments there were no significant changes in circulating plasma volume, serum proteins, or blood counts. There were slight but definite increases in the red cell volume in 11 out of 14 experiments. The lack of change in plasma volume was attributed to a summation of the two adjustment factors described above which tend to neutralize each other.

4. In steady states the increase in pulse rate correlates well with rises in rectal temperature. If the plasma volume increases, the rise in pulse rate per degree rise in rectal temperature is less than in those subjects whose plasma volume remains unchanged or decreases.

5. A considerable quantity of fluid can be requisitioned from the tissues and evaporated from the blood plasma without affecting the circulating blood volume.

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LOCALIZATION OF THE MEDULLARY RESPIRATORY CENTERS IN THE MONKEY[1]

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Although it has been accepted that mechanisms for the neural control of respiration are situated in the reticular formation of the medulla oblongata (Finley, 1931; Cordier and Heymans, 1935), only recently have attempts been made, by modern methods, to localize the respiratory region more precisely and to examine the question of its functional subdivision. In 1939 Pitts, Magoun and Ranson, studying responses in the cat to brain stem stimulation with the Horsley-Clarke instrument, outlined a reactive portion of the reticular formation, extending caudally from the level of the facial nucleus over the cephalic four-fifths of the inferior olivary nucleus. Within this area they defined 2 discrete divisions, from which, respectively, coördinated inspiratory and expiratory acts could be elicited. These were designated the inspiratory and expiratory centers. Brookhart (1940), employing slightly different technics and somewhat lower intensities of electrical stimulus, was unable to confirm the existence of these centers in the dog and challenged the concept of functional localization within the respiratory-reactive part of the reticular substance.

The objections raised by Brookhart (1940) to the technical procedures utilized by Pitts, Magoun and Ranson (1939) have been considered by Pitts (1941) and Magoun and Beaton (1941). In addition, in order to study the possibility, implicit in Brookhart's results, of genus and order differences in the organization of central respiratory mechanisms, as well as to examine the medullary regulation of respiration in a form more closely related phylogenetically to man, the present study on the monkey was undertaken.

Methods. Fourteen monkeys (Macaca mulatta), averaging slightly less than 3 kgm. in body weight, were used. Some were normals; others had been previously subject to acute explorations or chronic lesions of the hypothalamus. No dissimilarities in the respiratory responses could be detected between members of the normal and the operated series. The animals were anesthetized with nembutal, 15 to 25 mgm. per kilogram of

[1] Aided by a grant from the Rockefeller Foundation.

[2] Medical Fellow of the National Research Council.

body weight, injected intravenously and occasionally supplemented with ether during surgical procedures; or with urethane, 0.8 mgm. per kilogram of body weight, injected intraperitoneally. There were no discernible differences of respiratory reaction between the 2 anesthetic groups.

The medulla of each animal was systematically explored by use of the stereotaxic instrument of Horsley and Clarke, after the technic of Ranson (1934). Insertion of the electrodes in the customary vertical plane of the machine was unsatisfactory, because of inability to reach the midline, a difficulty apparently due to lateral deflection of the electrodes by the tentorium cerebelli. Therefore, recourse was had to the posterior electrode carrier, and the medulla was approached from behind. The plane thus afforded is oblique; points on the ventral surface of the brain are 2 mm. further rostral than they would be on a conventional transverse section made at the same dorsal level. However, this amount of inclination is not enough notably to distort the outlines of medullary structures or impede their identification. Operative exposure was achieved by enlarging the foramen magnum with rongeurs as far forward as the transverse sinus and incising the dura mater.

Bipolar electrodes of enameled nichrome wire were used, the exposed tips being separated from one another by 0.2 mm. or less along the axis of the electrodes. The stimulation, that of thyratron regulated condenser discharges, was similar to that used on the cat (Pitts, Magoun and Ranson, 1939; Magoun and Beaton, 1941). The period of excitation was fixed at 15 seconds. A frequency of 300 per second was found suitable, though responses to other frequencies were also analyzed. Intensities of stimulus were varied from 0.9 to 30.0 volts, a strength of 8.7 volts being employed for routine stimulation.

All points stimulated were located on Weil-stained serial sections cut in the plane of the punctures. A series of projection tracings was prepared from sections taken at ½ mm. intervals on the formalin fixed brain, and the stimulated points grouped according to level and plotted on the diagrams. Figure 2 consists of 6 levels selected from this series.

Respiration was recorded as a kymogram by cannulating the trachea, attaching to the cannula a small spirometer built in the form of the familiar basal metabolic rate machine, and fastening a pointer to the moving chamber of the spirometer. Carbon dioxide was absorbed by soda lime in a sleeve placed between the cannula and the spirometer, and oxygen was added as needed. Such a closed system, needing intermittent refilling, yields sloping records; those presented in figure 1 have been trimmed to conserve space.

Observations. As in the cat, many varieties of response were obtained, involving all combinations of changes in amplitude, rate and level of respiration. The concern of this study is with the latter, and *only*

those reactions showing a definite inspiratory or expiratory tendency have been considered. They have been classified into inspiratory and expiratory

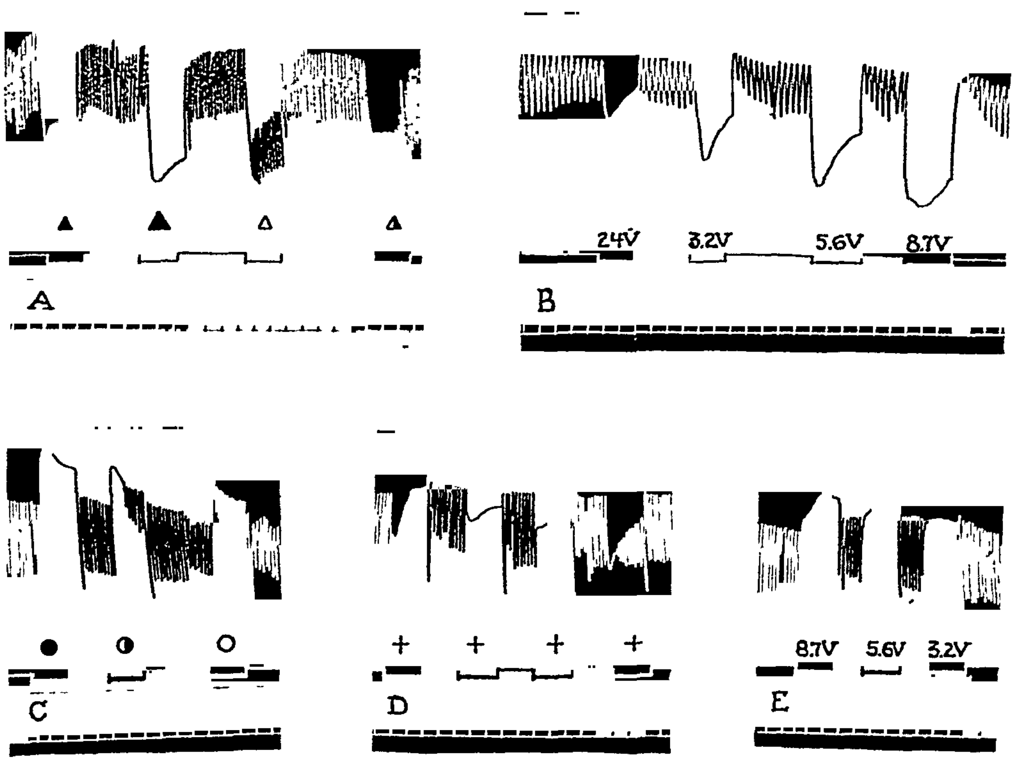

Fig. 1. Spirometer tracings of respiratory reactions to stimulation of the medulla. In A, C and D stimulus strength was 8.7 v. at 300/sec. In all records the time line carries intervals of 6 seconds and the duration of excitation is indicated by the signal magnet. Inspiration is represented by the downstroke, expiration by the upstroke. The symbols accompanying the responses in A, C and D are those used in plotting the localizing diagrams of figure 2. A. Representative inspiratory responses: sustained inspiratory apnea involving an increase in the volume of inspired air of less than 25 cc., a small solid triangle; sustained inspiratory apnea involving an increase of more than 25 cc., a large solid triangle; respiration continued at an augmented inspiratory level, an open triangle; inspiratory apnea breaking into rhythmic respiration before the end of stimulation, a half-filled triangle. B. Increase in the amplitude of inspiratory apnea produced by successively higher voltages administered at a single inspiratory-reactive point. C. Representative expiratory responses: sustained expiratory apnea, a solid circle; expiratory apnea interrupted by periodic respiration during the application of stimulus, a half-filled circle; respiration continued at a heightened expiratory level, an open circle. D. Types of midpositional responses, i.e., apneas midway between the normal expiratory and inspiratory peaks or decreases in amplitude between these levels. E. Decreases in the degree of expiratory response as produced by successive diminution of the strength of stimulus delivered at a representative expiratory-reactive site.

responses of different degrees, midpositional responses and negative responses, as illustrated in figure 1 (A, C and D) and defined in its legend.

The similarity of the reactions in the monkey to those previously described for the cat (Pitts, Magoun and Ranson, 1939; Magoun and Beaton, 1941) renders exhaustive discussion of them unnecessary. The only distinction of any possible moment between the two animals was in the amount of

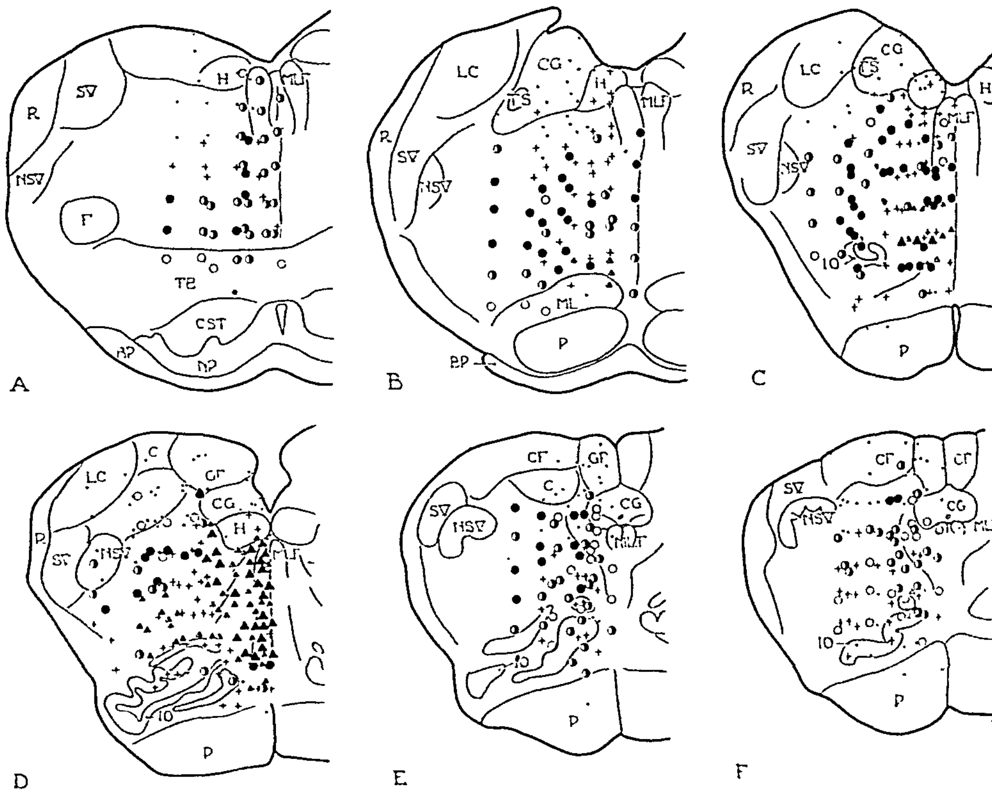

Fig. 2. Six levels through the medulla of the monkey, selected from the complete series, on which have been plotted the responses obtained to 8.7 v. stimulation at 300/sec. Symbols are those explained in figure 1: triangles for inspiratory responses; circles for expiratory; and crosses for midpositional reactions. Negative points are represented as dots. Distances between levels: A–B, 1 mm.; B–C, ½ mm.; C–D, 1 mm.; D–E, 1 mm.; E–F, ½ mm. Number of animals represented at each level: A, 2; B, 3; C, 3; D, 4; E, 4; F, 4. Brachium pontis, *BP*; cuneate nucleus, *C*; fasciculus cuneatus, *CF*; central grey, *CG*; corticospinal tracts, *CST*; facial nucleus, *F*; fasciculus gracilis, *GF*; hypoglossal nucleus, *H*; inferior olivary complex, *IO*; lateral cuneate nucleus, *LC*; medial lemniscus, *ML*; medial longitudinal fasciculus, *MLF*; nuclei pontis, *NP*; nucleus of the spinal tract of the fifth cranial nerve, *NSV*; pyramid, *P*; restiform body, *R*: spinal tract of the fifth cranial nerve, *SV*; trapezoid body, *TB*: tractus solitarius, *TS*.

air involved. In general, the apneic response to stimulation, especially when it was inspiratory, involved a smaller volume of oxygen in the monkey than in the cat. The reason for this is not clear; rough measurements of lung capacity have not revealed any significant inequalities between the two animals.

As in the cat, 2 separate areas were delineated, from which, respectively, inspiratory and expiratory apneas were consistently elicited. The distribution of responses (to 8.7 volts stimulation) is illustrated in figure 2 on 6 selected sections which cover the entire antero-posterior extent of the respiratory-reactive area. Levels 1 mm. rostral to that of figure 2 A and 1 mm. caudal to that of figure 2 F gave no sustained cessations of respiration.

The inspiratory field (fig. 2 B-D) extends 2 mm. rostro-caudally in the reticular formation of the medulla. Cephalically, slight responses are met with at the very caudal end of the pons, immediately above the medial lemniscus (fig. 2 B). The first maximal inspirations occur ½ mm. analward where they are found lying medially to the rostral extremity of the inferior olive (fig. 2 C). Proceeding caudad, the inspiratory region rapidly enlarges both dorsally and laterally and at the level of figure 2 D reaches the midline and the dorsal limits of the reticular formation. At this level sustained inspirations are obtained from a field reaching from a point between the 2 inferior olives to one immediately beneath the hypoglossal nucleus and spreading laterally some 3 mm. from the midline in the area dorsal to the olive. A level (not illustrated) ½ mm. caudal to that represented by figure 2 D demonstrates a similar distribution of responses except that the lateral reach of the center is decreased to 2 mm. One millimeter posterior to level D, inspiratory responses abruptly disappear (fig. 2 E). Thus the inspiratory area lies dorsally and medially to the rostral half of the inferior olivary nucleus in a field which at its rostral end is basally and medially located and which, toward its caudal end, gradually expands both dorsally and laterally.

The expiratory field (fig. 2 A–F) surrounds the inspiratory, lying rostrally, laterally and caudally to the latter, and also dorsally to it except at that level where the inspiratory area reaches the hypoglossal nucleus (fig. 2 D). In addition, scattered expiratory reactions can be elicited from sites beneath the inspiratory center (fig. 2 C and D). Maximal expiratory apneas are obtained from the midline only ahead of the inspiratory region (fig. 2 B and C), and caudal to the latter area expiratory responses are generally weak and dispersed (fig. 2 E and F). The greatest lateral extent of the expiratory center is 4 mm. from the median raphé. For purposes of rough description, the expiratory field can be said to be coextensive with the reticular formation from a level slightly more than 1 mm. ahead of the rostral end of the inferior olive to the latter's caudal extremity, except for the compact area occupied by the inspiratory center. On the whole expiratory responses are more disseminated than are inspiratory responses.

The relation of the respiratory fields to familiar dorsal landmarks of the brain stem is shown in figure 3, which is a projection of these fields,

as reconstructed from the complete set of levels, onto the floor of the 4th ventricle. The regions outlined are those giving sustained responses to 8.7 volts stimulation. The projection was made, not in the plane of the sections of figure 2, but in the true transverse plane.

Close inspection of figure 2 reveals a suggestive apportionment of what are characterized as "midpositional points," i.e., points yielding apnea anywhere between normal expiratory and inspiratory levels or a reduction of respiratory excursion between these limits. These midpositional responses (plotted as crosses), though somewhat sporadic in distribution, are clumped in 2 particular situations. First, they are found skirting the

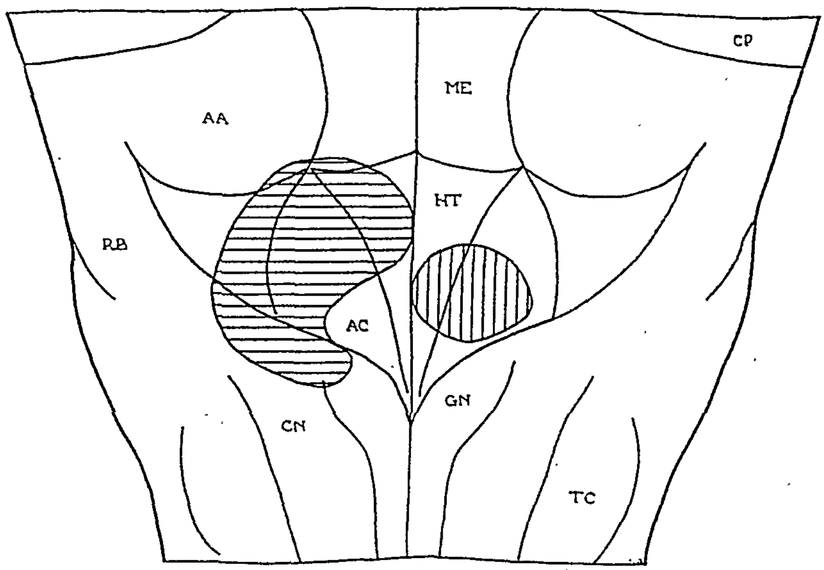

Fig. 3. Dorsal view of the lower brain stem of Macaca mulatta with cerebellum removed. The areas of sustained inspiratory apneas (on the right; vertical lining) and sustained expiratory apneas (on the left; horizontal lining) are shown projected onto the floor of the 4th ventricle. To avoid overlapping the two regions are shown on different sides of the brain stem. Area acoustica, *AA*; ala cinérea, *AC*; cuneate tubercle, *CN*; cerebellar peduncle, *CP*; clava, *GN*; hypoglossal trigone, *HT*; medial eminence, *ME*; restiform body, *RB*; tuberculum cinereum, *TC*.

outer boundary of the expiratory field (fig. 2 B, C and F), (they were elicited rostrally and caudally to the expiratory area at levels not illustrated). In this locus these were usually midpositional apneas tending toward the expiratory side and were mingled with expirations which broke into rhythmic respiration and continued respirations of increased expiratory height. In these 3 types of reaction the points stimulated may not have been sufficiently close to the center of concentration of reactive elements to produce sustained responses. The second location of midpositional points is a zone between the inspiratory and expiratory fields (fig. 2 C and D). Here such responses were midpositional apneas, some

of which moved during the period of stimulation toward the expiratory side of the kymograph tracing, some toward the inspiratory. This band, intermediate between the inspiratory and expiratory centers, may represent a situation in which roughly equal numbers of inspiratory and expiratory elements are subjected to excitation. The midpositional reactions of figure 1 D were obtained as the electrode passed from an expiratory region to an inspiratory, and the shift of the level of respiration from the expiratory to the inspiratory side is clearly evident.

In general, stimulation of medullary structures other than the reticular substance did not yield the respiratory responses in question. When they were obtained from other formations, it was from those directly contiguous with the main reactive fields. These aberrant points were diminished in number when stimulus strength was reduced and they are presumably due to current spread to the more specifically sensitive areas.

Investigation of changes in the types and localization of responses with alteration in stimulus strength has not been a primary aim of this study. So that the topography in the monkey might be compared with that delineated for the cat (Pitts, Magoun and Ranson, 1939), routine stimulation was done at 8.7 volts. However, many experiments were done with lower stimulus strengths (0.9 to 5.6 volts); the results confirmed the conclusions drawn from the investigation of low voltage responses in the cat (Magoun and Beaton, 1941). In addition, reactions to a higher voltage (13.7 volts) were examined. Reduction of the intensity of excitation caused some shrinkage of the respiratory fields as well as some diminution in the magnitude of responses, especially those responses found at the peripheries of the fields. Yet, many sustained inspirations were obtained at 2.4 volts, some at 0.9 volts. Sustained expirations were procured by voltages down through 3.2 volts. The topography of response was in no way changed by the use of lower voltages, and in no instance was the direction of response altered by a voltage decrement or, for that matter, by a frequency change. Figure 1 (B and E) illustrates representative alterations in the magnitude of inspirations and expirations with a succession of voltages. It also shows that the volume of inspiratory apnea was usually greater than that of expiratory apnea. These findings, even to the threshold voltage values, are in striking concordance with results in the cat (Magoun and Beaton, 1941).

Two macaques were subjected to medullary stimulation 2 weeks after a left hemisection at the pontile level. No significant differences were found between responses elicited at the same locations on the 2 sides of the brain, and almost maximal inspirations and expirations were obtained from both sides in each animal.

Discussion. The localization of inspiratory and expiratory fields has been found to be as precise in the monkey as in the cat. The delimitation

of these fields by the use of "high voltages" (8.7 volts) is deemed entirely permissible because of evidence elsewhere presented (Pitts, 1941; Magoun and Beaton, 1941). By having outlined these areas with relatively high stimulus strengths, any error would seem to be in the direction of diffusion, especially since employment of lower voltages has given some contraction of the regions with maintenance of their general topography and relationships.

The anatomical locations of the respiratory centers in the monkey correspond in general with those of the cat. The topographical differences in the two animals do not seem too great to be explained by very evident variance of medullary structures in these members of separate taxonomic orders. The inspiratory region is less and the expiratory region more disperse in the monkey than in the cat. The inspiratory area of the cat lies ventrally to the expiratory throughout the extent of their anteroposterior overlap, while in the monkey the inspiratory field, at its caudal extremity, rises high in the midline and no expiratory responses are obtained dorsally to it. Also, in the cat, expirations cannot be elicited from loci caudal to the inspiratory center as they can in the monkey. Some of these dissimilarities in the topography of the excitable regions in the two animals may perhaps be related to the larger size of the inferior olive in the monkey and the consequent alteration in the shape of the reticular substance.

The cephalic and caudal boundaries defined for the respiratory area in the monkey agree with the limits set for the dog by tapping from the brain stem amplified potentials showing a respiratory rhythm (Gesell, Bricker and Magee, 1936). The inability to demonstrate discrete expiratory and inspiratory centers in the dog (Brookhart, 1940) contrasts with the success now achieved in both the cat and monkey.

A comparison of the location of the respiratory centers in monkey and man cannot be made with any precision, due to the lack of exact information on the site of the centers in man. A rough accordance is indicated by reported cases of clinical neurogenic respiratory failure (Finley, 1931; Nordmann and Müller, 1932) in which the lesions were in the reticular formation overlying the inferior olive.

The finding that left pontile hemisection in 2 animals did not affect the bilateral distribution or magnitude of response is regarded as a further contribution to other evidence at hand (Magoun and Beaton, 1941) that the respiratory reactions elicited by medullary stimulation should probably not be attributed to the excitation of clustered direct afferents to the reticular formation.

SUMMARY

Circumscribed electrical stimulation of the medullas of 14 monkeys by means of the Horsley-Clarke technic has revealed the existence of 2 dis-

crete regions, from one of which sustained inspiratory apnea, from the other sustained expiratory apnea were consistently obtained. The inspiratory field is located dorsally and medially to the rostral half of the inferior olive. The expiratory field surrounds the inspiratory, lying rostrally, caudally, laterally and to some extent dorsally to the latter. Successive decreases in stimulus strength down to threshold values did not change the character or topographical arrangement of the responses. The anatomical localization is in general agreement with that found previously for the cat. In 2 monkeys with pontile hemisections, there was no diminution of reaction as compared with normal animals nor any difference in the responses obtained from the 2 sides of the medulla.

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RESPIRATORY RESPONSES FROM STIMULATION OF THE MEDULLA OF THE CAT[1]

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In a study of the respiratory responses to stimulation of the medulla of the cat, Pitts, Magoun and Ranson (1939) described an excitable reticular field from whose dorsal and ventral parts expiratory and inspiratory reactions were respectively elicited. The results appeared to extend our knowledge of the location and functional organization of the respiratory center, but experiments on the dog led Brookhart (1940) to conclude that the respiratory responses described could be obtained only by high voltage stimulation whose great range of spread prevented localization of reactive regions. Emphasis has recently been placed by Gesell (1940) upon an alternative explanation of the responses from medullary stimulation, i.e., that they result from activation of afferent fibers exerting a predominantly inspiratory or expiratory influence upon respiration.

It seemed desirable, therefore, to review the respiratory responses from the medulla of the cat with reference to the voltage and range of spread of stimulating current and the distribution of reactive areas. In addition responses have been examined after chronic ablation procedures designed to eliminate certain afferent pathways whose excitation might be responsible for the responses.

METHODS. In lightly anesthetized cats, respiration was recorded with a spirometer and stimulation or the production of lesions was carried out with the Horsley-Clarke technic (Ranson, 1934). Unless noted, stimuli consisted of thyratron regulated condenser discharges at 300 per second with voltages between 0.9 and 8.7.

RESULTS. *Respiratory responses to stimulation.* The responses to which attention was directed are shown in figure 1; at the left are a group of expiratory responses elicited from the dorsal reticular formation and at the right are a group of inspiratory responses obtained from the ventral reticular formation. In each group, on increasing the voltage of stimulation, the increased magnitude of reaction is apparent both in the extent

[1] Aided by a grant from the Rockefeller Foundation.

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to which the response dominates over regular breathing and in addition by the increased amplitude of expiration or inspiration.

Distribution of reactive areas. Data on the distribution of excitable areas for these respiratory responses on altering the voltage of stimulating current have been obtained by activating a series of points in transverse levels through the excitable region, and at each point recording the responses to voltages between 0.9 and 7.4. This range was chosen because results obtained with 1 volt were those to which Brookhart (1940) attached significance, while stimuli of 8 volts were used routinely by Pitts, Magoun and Ranson (1939).

The results of an experiment are shown in figure 2. A series of points were stimulated in levels I and II through the excitable region and on

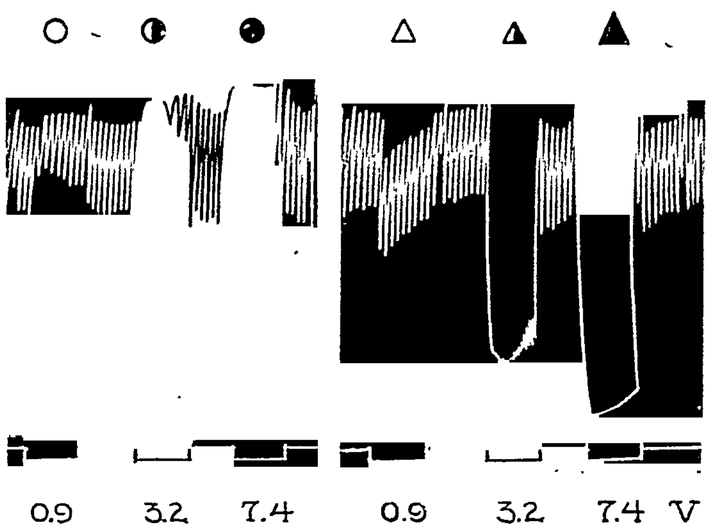

Fig. 1. Spirometer tracings showing weak, intermediate and marked expiratory (left) and inspiratory (right) responses to 15 seconds' stimuli at the voltages indicated. The symbols above designate the type of response in figure 2.

3 copies of each level are shown the site and magnitude of reactions to voltages of 0.9, 3.2 and 7.4. An examination of the data presented in the figure reveals that in the case of both the expiratory and the inspiratory fields the effects of increasing the intensity of stimulation, within the range of 1 to 8 volts, is primarily to augment the magnitude of the responses obtained and not appreciably to increase the distribution of the reactive areas. The excitable reticular formation encountered by Pitts, Magoun and Ranson (1939) is evident with each of the voltages used and its subdivision into dorsal expiratory and ventral inspiratory zones is clearly apparent.

Threshold. A number of observations, some of which are indicated in figure 2, permit the conclusion that with thyratron regulated discharges

at 300 per second, the threshold for marked inspiratory responses is from 1 to 2 volts, that for marked expiratory reactions is from 3 to 4 volts. Similar low thresholds were found using 60 per second sine wave stimuli.

Effect of lesions at the point of stimulation. The repetition of stimuli after producing lesions around the point of stimulation is a method used by Brookhart (1940) to estimate the distance of current spread. The

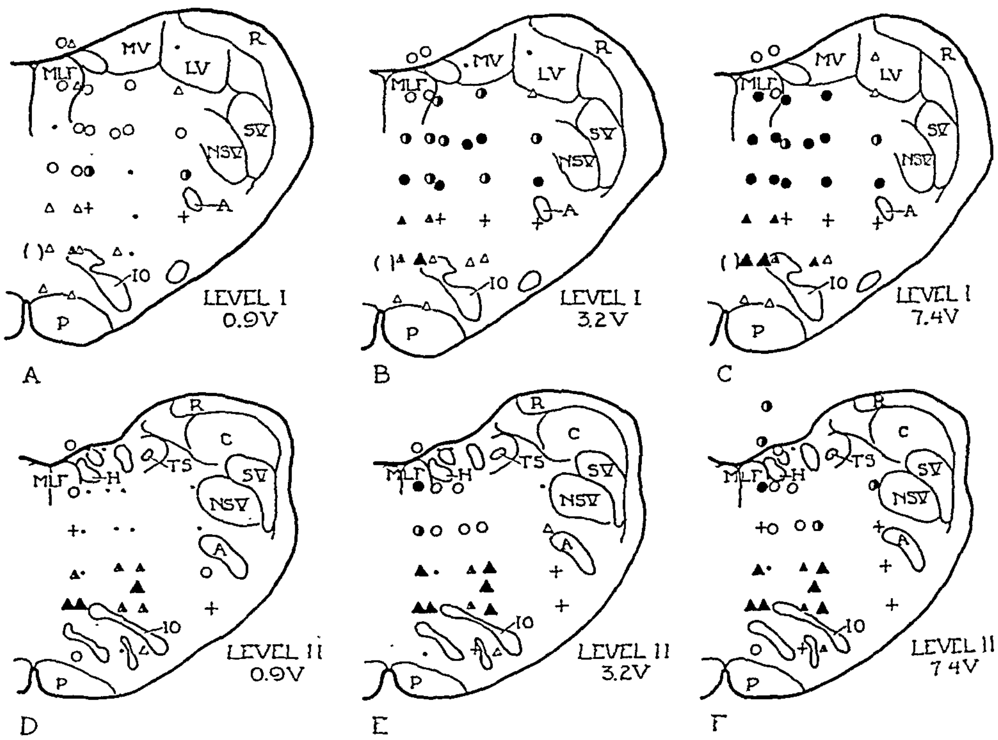

Fig. 2. Transverse sections of the medulla of the cat, showing the respiratory responses obtained at 2 levels of stimulation with voltages of 0.9, 3.2 and 7.4. The results from both halves of the medulla are shown on the right side. Expiratory responses are indicated by circles and inspiratory reactions by triangles as in figure 1. Small and large filled triangles indicate marked inspiratory responses of less and more than 75 cc., respectively. Crosses show mid-positional changes, and dots, negative points. Abbreviations are as follows: nucleus ambiguus, *A*; lateral cuneate nucleus, *C*; hypoglossal nucleus, *H*; inferior olive, *IO*; lateral vestibular nucleus, *LV*; medial longitudinal fasciculus, *MLF*; medial vestibular nucleus, *MV*; nucleus of spinal fifth tract, *NSV*; pyramid, *P*; restiform body, *R*; spinal fifth tract, *SV*; tractus solitarius, *TS*.

effects of a number of lesions were studied in the present investigation and the results of an experiment are shown in figure 3.

An electrode was inserted into the ventral reticular formation and the inspiratory responses to stimuli between 0.9 and 8.7 volts were recorded (fig. 3 A). After the production of a lesion, the same voltage series of stimuli was repeated and the responses obtained in the first minutes were

either abolished or greatly reduced (fig. 3 B). Improvement occurred and the responses obtained 18 and 32 minutes after the lesion reflected the development of a rather steady state (fig. 3, C, D). The final response elicited with 8.7 volts (fig. 3 D) was equal in magnitude to that obtained initially with 2.4 volts (fig. 3 A), amounting to an over-inspiration of 60 cc.

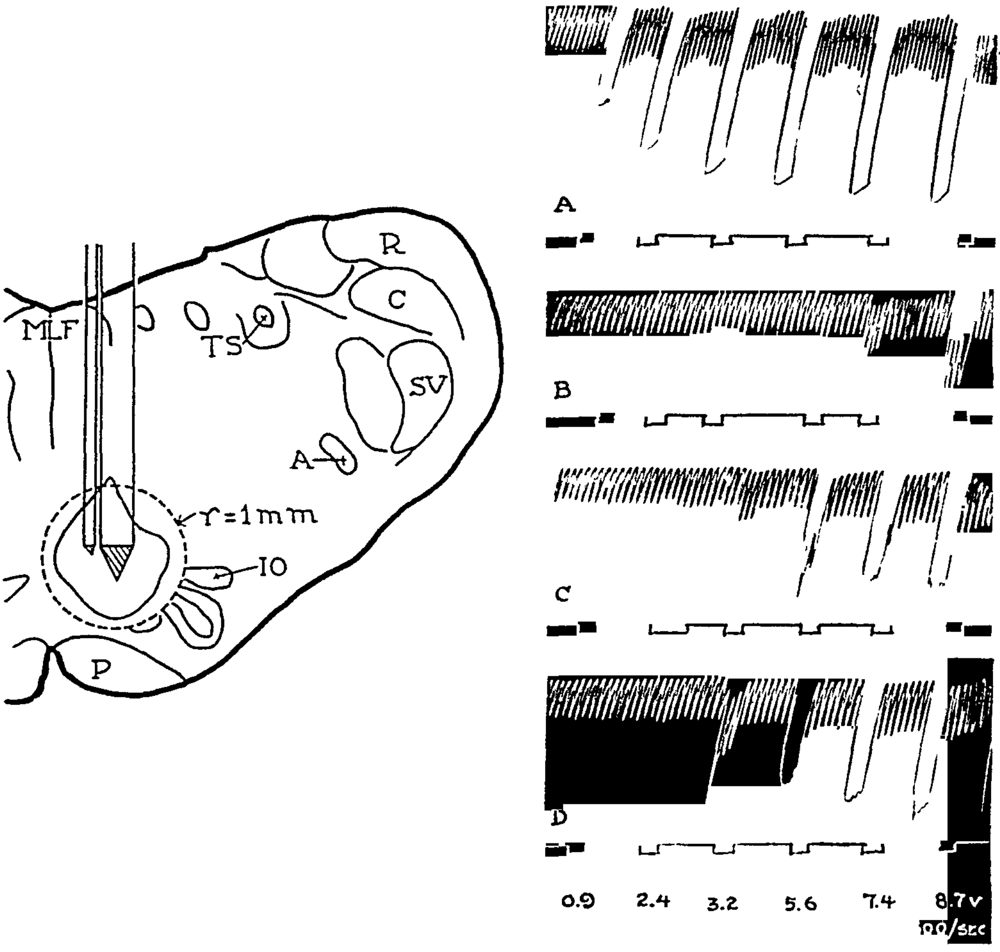

Fig. 3. Transverse section of the medulla (left), showing the position of electrodes and lesion. The dotted circle inclosing the lesion has a radius of 1 mm. drawn to scale. At the right are spirometer tracings of inspiratory responses obtained before (A), and 1 minute (B), 18 minutes (C), and 32 minutes (D) after the lesion. The voltages of the 15 second stimuli are indicated.

Since the response obtained initially with 2.4 volts, unlike those to higher voltages, remained abolished after the lesion, it was presumably elicited entirely from the volume of tissue destroyed, which extended in every direction some 0.5 mm. from the surface of the electrodes. The final equivalent response obtained with 8.7 volts would have been elicited

from an equal volume of tissue if a linear relation existed between the magnitude of response and the amount of tissue activated. This tissue if distributed evenly around the margins of the lesion would extend some 0.7 mm. from the surface of the electrodes, and according to this reasoning a stimulus of 8.7 volts should spread about 0.7 mm.

Infrequently a transient augmentation of response was found in the early period after producing a lesion. Because the magnitude of reaction does not vary greatly when stimuli are repeated at intervals over a long period, a sudden increase sometimes found after making a lesion (see also Brookhart, 1940) suggests that some factor associated with the lesion has increased the usual range of current spread or augmented the excitability of the surrounding tissue. Such a suggested alteration may regularly be present and the responses obtained after lesions may reflect the balance struck between it and response reducing factors. In our opinion further study of the lesion method should be undertaken before using it as a simple test of current spread.

Partial deafferentation. Attention may here be directed away from questions of method to a consideration of the possibility that the responses obtained from medullary stimulation (fig. 1) are the result of activation of afferent fibers exerting an influence upon respiration. The results of Pitts, Magoun and Ranson (1939) showed that these responses were not elicited from the nucleus of the tractus solitarius or the posterior column nuclei where they might have been expected to be obtained, were excitation of glossopharyngeal, vagal or thoracic proprioceptive afferents the basis of their production.

To investigate the possibility that unknown afferent pathways from the vagus and glossopharyngeal nerves to the reticular formation were responsible for the results, the distribution, magnitude and threshold of responses from the 2 halves of the medulla were compared 2 weeks after intracranial section of the glossopharyngeal and vagal rootlets on 1 side. With minor variations, which were without regard as to laterality, all features of the reactions on the 2 sides showed close similarity.

In each of 2 other cats, the respiratory responses from the 2 halves of the medulla were compared after chronic hemisection of the spinal cord at C 1. Exploration of 4 levels was completed and in one level a slight restriction of the inspiratory field on the side of hemisection was noted and the amplitudes of both expiratory and inspiratory responses were smaller on this side. In the other 3 levels no significant difference in the features of the responses from the 2 halves of the medulla could be observed.

These results, which should be combined with those obtained after chronic pontile hemisection in the monkey (Beaton and Magoun, 1941), permit the statement that the responses to medullary stimulation are not

attributable to the activation of a direct, ipsilateral, afferent pathway approaching the reticular formation either from the pons, or from the spinal cord, or by way of the vagus or glossopharyngeal nerves.

SUMMARY

The respiratory responses obtained from stimulation of the medulla of the cat have been reviewed with reference to the voltage and range of spread of stimulating current and the distribution of reactive areas. The results do not support the view that these responses are dependent upon the activation of large, indiscriminately situated regions of the medulla by widely spreading stimuli, but indicate, on the contrary, definite areas responsive to locally acting excitation.

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THE DIFFERENTIATION OF RESPIRATORY CENTERS[1]

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While it is generally agreed that the basic neural elements necessary for integration and regulation of respiration are to be found within the reticular formation of the medulla oblongata caudal to the level of entrance of the eighth nerves (Cordier and Heymans, 1935), no concept of the morphological organization of this so-called respiratory center is universally accepted. Gesell, Bricker and Magee (1936) studying action potentials tapped from the lower brain stem, and Brookhart (1940) stimulating the same region with shocks of near threshold intensity, conclude that the neurones controlling inspiratory and expiratory activities are diffusely intermingled and show no grouping into discrete centers. On the other hand Nicholson (1936) observing respiratory modifications to local cooling of the floor of the fourth ventricle, and Pitts, Magoun and Ranson (1939a) stimulating the brainstem with shocks of moderate intensity, conclude that the respiratory center may be divided into a dorsal expiratory and a ventral inspiratory center. The latter investigators localized the inspiratory center to the ventral reticular formation overlying the cephalic four-fifths of the inferior olivary nucleus, and the expiratory center to the dorsal reticular formation, dorsal to, slightly cephalic to, and cupped over the cephalic end of the inspiratory center.

Brookhart (1940) and Gesell (1940) have criticized this localization along three lines: 1, excessive physical spread of stimulating current; 2, with such spread a functionally meaningless differentiation of the respiratory center into inspiratory and expiratory divisions; 3, too all inclusive criteria for differentiation of centers which might mask simultaneous activation of antagonistic elements.

Although various indirect lines of evidence could be brought forward in answer to the above criticisms, it was felt that the importance of accurate knowledge of the morphology of the respiratory center justified a reinvestigation of the problem of differentiation. Accordingly experiments were

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[2] Littauer Fellow of Neurophysiology.

designed to provide more or less direct information on each of the points in question.

The measure of stimulus spread. Methods proposed to assess physical spread of stimulating current within the brain have been based either upon the distance the electrode tips must be advanced to abolish or reverse a motor response (Pitts, Magoun and Ranson, 1939a; Hinsey, 1940), or upon the size of an electrolytic lesion necessary to abolish the response at a given placement of the electrodes (Brookhart, 1940). These methods give only qualitative results; the latter, in addition, is complicated by a marked change in physical properties of the tissue surrounding the lesion (Magoun and Beaton, 1941).

The experimental preparation used to provide more quantitative data on the problem of current spread within the brainstem of the cat is illustrated in diagrammatic form in figure 1. Fibers in the sensory frontal branch of the trigeminal nerve have their cell bodies in the Gasserian ganglion, enter the brain at a pontile level, and turn sharply caudad in the bulbar trigeminal tract, located laterally throughout the medulla. Collaterals are given off in the nucleus and after synapsis the secondary trigeminal pathways continue. If recording electrodes are placed on the frontal nerve this primary sensory system may be activated in reverse by stimuli applied to the tract through bipolar needle electrodes oriented in the Horsley-Clarke stereotaxic instrument.[3] Stimuli are effective only when they are applied to the tract itself or to the collaterals as they pass into the nucleus of the tract located just medially. Activation of the secondary pathways leads to conduction only to the cell body in the nucleus, since excitation does not pass the synapse the wrong way.

Stimuli applied to the tract were brief, thyratron regulated condenser discharges (time constant approximately 0.1 m. sec.) at an intensity of 8 volts[4] and at a frequency of 100 per second. A bridge transformer between stimulator and electrodes reduced stimulus artifact. Potentials of the frontal nerve were amplified by a condenser coupled amplifier and applied to a cathode ray oscillograph. The cathode ray sweep was synchronized so as to produce a standing wave which could be measured or photographed. The height of the potential record serves as a rough indication of the number of fibers which lay within a zone about the electrode tips where the stimulus intensity was above threshold. On the left of figure 2 is a projected tracing of a Weil stained section of the cat brain showing the position of the needle electrode. The interruptions of the black line indicate the

[3] The type of needle electrode used and the method of histological identification of structures stimulated were described in our original communication (Pitts, Magoun and Ranson, 1939a).

[4] Repeated checks of voltage at the electrodes were made during each experiment by switching directly from electrode terminals to oscillograph.

position of the needle tip at each half millimeter as it was lowered into the medulla. On the right are records of the potentials evoked in the frontal nerve. No response was obtained until position 1 was reached where a minute deflection occurred. The deflection which occurs in all records at

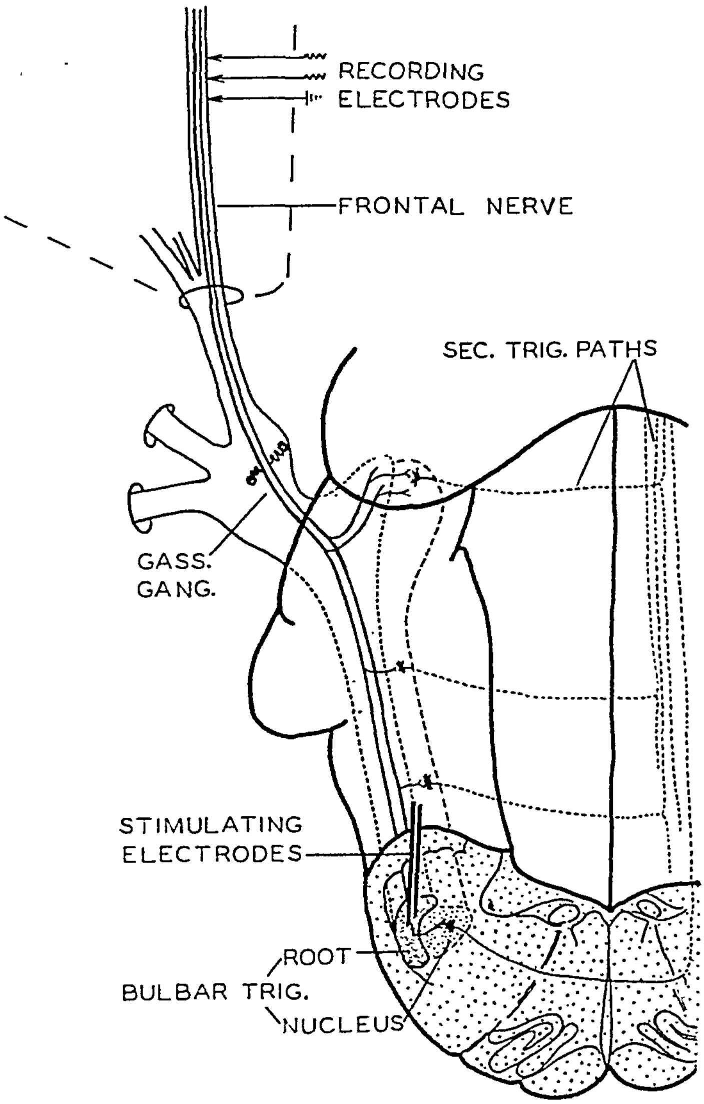

Fig. 1. Diagrammatic three-dimensional sketch of the lower brainstem of the cat with cerebellum removed, to illustrate the use of the frontal-nerve-bulbar-tract system to assess stimulus spread; see text for details.

the beginning of the sweep is stimulus artifact. Lowering the electrode $\frac{1}{2}$ mm. from position 1 to 2 increased the response tremendously. Penetration of the needle tip was stopped at this point in order to mark the position accurately.

A similar experiment is shown in figure 3 except that two intensities

of stimuli were applied at each level of electrode penetration, namely, 8 and 16 volts. No response was obtained until the electrode tips penetrated

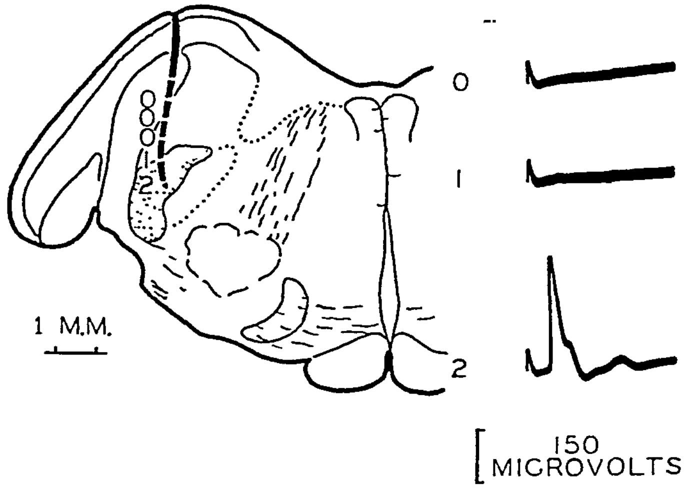

Fig. 2. Potentials recorded from the frontal nerve on lowering of the stimulating electrode in successive half-millimeter steps into the bulbar trigeminal root (stippled). Stimulus, 8 volts; initial deflection of the sweep, stimulus artifact. Position of electrode tips at each level corresponding to potential record is shown by the break in the heavy black line representing the track of the electrode.

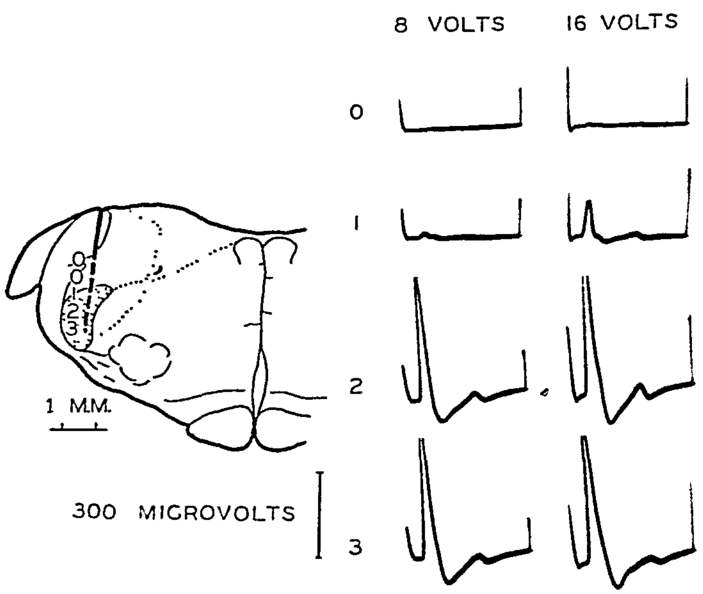

Fig. 3. Potentials recorded from the frontal nerve during stimulation at each half-millimeter level approaching and penetrating the bulbar trigeminal root with stimuli of two intensities, 8 and 16 volts. At position 3 the threshold was slightly less than 1 volt.

the tract and at position 1 (8 volts) a just perceptible response was evident. Increasing the stimulus to 16 volts increased the evoked potential some-

what, no doubt the result of increased current spread and consequent activation of more fibers within the tract. But lowering the electrode $\frac{1}{2}$ mm. at 8 volts produced a much greater increase in response than doubling the voltage at the previous position. At position 3 an even larger response was obtained both at 8 and 16 volts. At this position the intensity of the stimulus was lowered until a just perceptible response was obtained. The threshold was slightly below 1 volt. It is quite apparent then that stimulus intensity diminishes rapidly with distance from the electrode tips, falling to a value of about one-eighth or less a half millimeter away.

The measure of degree of localization possible. It may be concluded from the preceding results that at least theoretically the use of brief condenser shocks at an intensity of 8 volts should permit localization of structures within the brainstem with an error not greater than one-half millimeter,

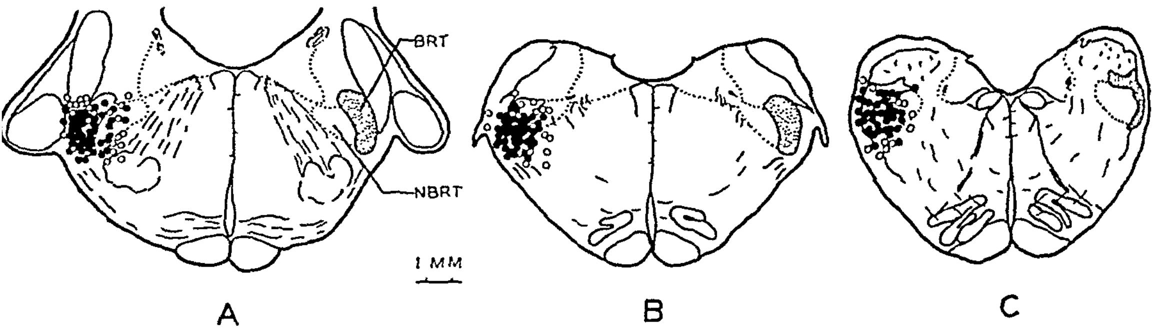

Fig. 4. Comparison of the bulbar trigeminal root as localized by stimulation (left) with its known morphology (right). Solid circles, frontal nerve potentials from one quarter to the maximum obtained in any given experiment; open circles, frontal nerve potentials of lesser magnitude. The dotted enclosure medial to the stippled root represents the nucleus of the bulbar trigeminal root.

possibly less. However, the bulbar-tract-frontal-nerve preparation is admirably suited for a direct comparison of physiological localization with known morphology, for the limits of the tract are fairly well defined.

A total of 10 experiments were performed exploring the medulla from the midline outward at a number of levels, with a stimulus intensity of 8 volts. The height of the frontal nerve response was measured on the face of the cathode ray tube at each position of the needle electrode. On the left side of the sections in figure 4 is shown the physiological localization of the bulbar trigeminal tract by the stimulation technique. On the right side in stippling is shown the true morphology of the tract. The dotted enclosure placed just medial to the tract represents the nucleus of the bulbar tract in which one would expect some collaterals from frontal nerve fibers. The solid circles in figure 4 represent evoked potentials of one-quarter or more of the maximum obtained in a given experiment, while the

open circles represent potentials of lesser magnitude. The least potential discernible in the frontal nerve at the amplification used amounted to about 0.5 per cent of the average maximum.

While the physiological localization is not a photographic likeness of the bulbar tract, it misses the boundaries on an average less than ½ mm., except medially, where collaterals pass into the bulbar nucleus. To this extent the physiological localization of the course of the primary trigeminal sensory axon is a more accurate one than that provided by inspection of a low power projection of the tract for the terminations of these axons in the nucleus are not apparent except at higher magnifications.

Although the original experiments localizing inspiratory and expiratory divisions of the respiratory center (Pitts, Magoun and Ranson, 1939a) had been performed with the same intensity and essentially the same wave form of stimulus as above, it was felt desirable to repeat them under exactly the same conditions. Hence a stimulus of 8 volts was applied through the

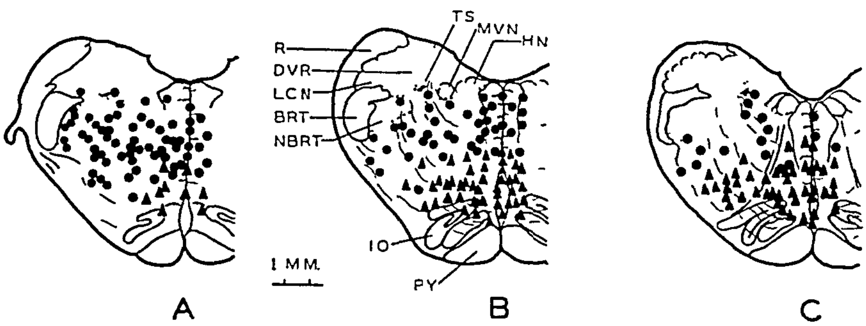

Fig. 5. Sections through the medulla oblongata of the cat at millimeter intervals to show the localization of the maximal inspiratory responses (triangles) and maximal expiratory responses (circles) described in the text.

same bridge transformer and the respiratory response measured by a closed spirometer circuit identical to that employed in the original investigation. A frequency of 240 per second was employed. The medulla was explored millimeter by millimeter within the general confines previously described for the center. Only the maximal responses are considered, namely, inspirations of 75 cc. or over plotted in figure 5 as triangles and expirations as great or greater than normal plotted as circles. Only inspirations and expirations maintained without interruption by rhythmic respiration for the duration of stimulation (12 sec.) were considered maximal.

The maximal respiratory responses shown in figure 5 represent the composite plot of results obtained on 6 cats at each level. Only the rostral 3 mm. of the respiratory center are shown. The dorsal distribution of expiratory responses and the ventral distribution of inspiratory ones are sufficiently obvious to require no further comment. A comparison of

sections A, B and C with sections C, D and E of figure 3 (Pitts, Magoun and Ranson, 1939a, p. 679) shows a remarkably close agreement. The only difference noted in this reinvestigation worth commenting upon is a more caudal extent of expiratory responses than was described previously, these reponses extending approximately as far caudally as the inspiratory ones, i.e., some 2 mm. caudal to section C of figure 5.

A consideration of the results presented in the preceding two sections leads to the conclusion that the stimulus is of threshold intensity only within a radius of one-half millimeter around the electrode tips; that in actual practice a structure may be localized within the brain to within at least one-half millimeter; and that the dorsal position of expiratory and ventral position of inspiratory responses must have a morphological basis.

Criteria for differentiation of the divisions of the respiratory center. Pitts, Magoun and Ranson (1939b) summarized the evidence that the maximal inspiratory and expiratory responses result from activation of two antagonistic and morphologically distinct divisions of the respiratory center, and these points need not be repeated here. It was felt that further evidence might be obtained by a study of the behavior of single respiratory motorneurones during stimulation of these centers, especially as concerns the possibility of simultaneous activation of excitatory and inhibitory reticular elements.

Accordingly, small strands containing one or more active fibers were carefully teased from the cut third phrenic root of the cat and potentials recorded by a condenser-coupled amplifier. Simultaneous respiratory tracings were obtained by connecting the tracheal cannula through a soda-lime tube to a 5-gallon bottle filled with oxygen. The small pressure changes of the closed system were measured by a light rubber optical tambour.

Figure 6 shows the potential records obtained from a small slip of the phrenic nerve in which, during the control strip of record, only a single fiber was spontaneously active, firing four impulses per inspiration. As the stimulating electrode was progressively lowered a millimeter at a time, through the dorsal and into the ventral reticular formation, stimulating at each level, records A, B, C and D were obtained. In records A and B obtained on stimulation of the dorsal reticular formation, the last of the four expected nerve impulses was clipped off and expiratory apnea maintained, for the duration of the stimulus. A millimeter shift of the electrode into the ventral reticular formation (B to C) altered the response from one of inhibition to excitation and in records C and D not only is the neurone of the control record active but at least 3 others as well. The position of the electrode at each level of the stimulation is shown in figure 7. Again, confirmation of the expiratory, or as concerns the phrenic, the inspirato-inhibitory function of the dorsal reticular formation, and the inspiratory

function of the ventral reticular formation, is evident. While inhibition of activity similar to that shown in A and B of figure 6 may be obtained by central stimulation of the vagus, the expiratory reactive area outlined in figure 5 does not conform with the course of primary sensory vagal neurones. That secondary sensory vagal fibers enter the dorsal reticular formation and there establish tertiary or higher order connections is highly probable. On the other hand, stimulation of the ventral reticular forma-

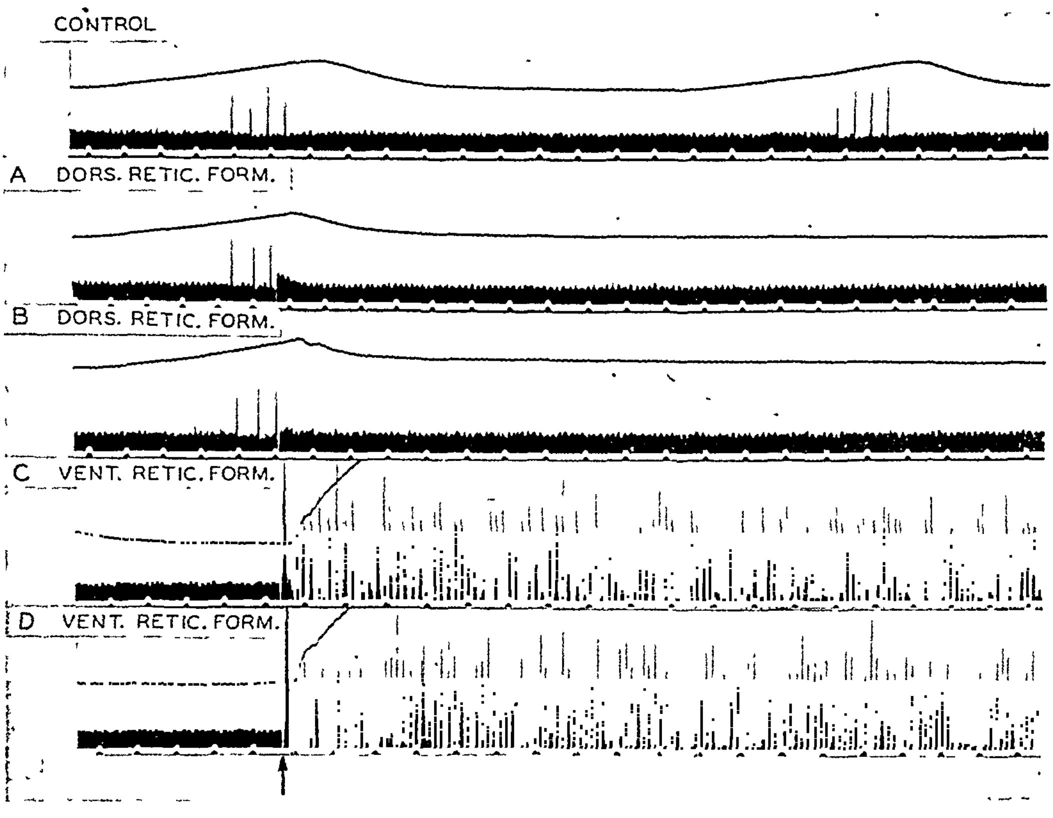

Fig. 6. Potentials recorded from a small slip of the phrenic nerve during stimulation of the medulla at the levels shown in figure 7. The time of application of the stimulus in records A to D is indicated by the arrow at the bottom of the figure. Stimulus, 8 volts, 240 per second; time, one-fifth second; upstroke of respiratory record indicates inspiration.

tion leads to a degree of excitation of phrenic neurones maintained for minutes which in our experience can be obtained from no afferent nerve. This of course does not mean that no elements pass through the dorsal reticular formation which have an excitatory effect. Indeed, second order vagal afferents, excitatory in nature, must send their processes across the dorsal to reach the ventral reticular formation. It merely means that the preponderance of elements of the dorsal reticular formation are inhibitory so far as the motor outflow of the phrenic is concerned, and that the pre-

ponderance of the ventral reticular elements are excitatory. Furthermore a localized stimulus, involving a millimeter cube of either the dorsal or ventral reticular formation, is capable of dominating the entire extent of that formation and inhibiting completely activity in the antagonist.

Discussion. The rather extensive control experiments cited provide a needed direct means of assessing current spread and degree of localization possible within the brainstem. They have shown that the field produced about the tips of bipolar needle electrodes by brief repetitive condenser discharges diminishes rapidly in intensity to one-eighth or less at a distance of $\frac{1}{2}$ mm. Utilizing such a method for physiological exploration, a well defined structure such as the bulbar trigeminal tract may be localized to within $\frac{1}{2}$ mm.

When this method was applied to the localization of the maximal respiratory responses described by Pitts, Magoun and Ranson (1939a), the original localization was confirmed in all essential details. One is therefore forced to conclude some morphological difference between the dorsal and ventral

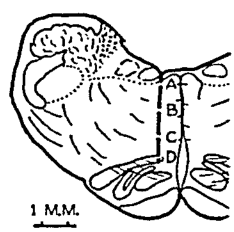

Fig. 7. Locus of the points stimulated in records A to D of figure 6. Note that A and B fall within the region defined as the expiratory center while C and D are in the inspiratory center.

reticular formations to account for the respective localizations of expiratory and inspiratory responses.

Stimulation of the ventral inspiratory center leads to repetitive activity of phrenic motor neurones, recruiting in new units not spontaneously active. Activation of the dorsal expiratory center, on the other hand, stops the spontaneous motor discharge of the phrenic. These results have been repeatedly confirmed and provide confirmatory evidence, not only for the dorso-ventral distribution of expiratory and inspiratory elements, but also for the localized character of the stimulus. If a level is stimulated in the region of junction of dorsal and ventral reticular formations (e.g., halfway between B and C of fig. 7), some admixture of inhibitory and stimulating effects is observed as might be expected.

The contrary results of Brookhart (1940) seem explicable in part on the near threshold intensity of stimulation which he used in the majority of his experiments. The failure to obtain a type of localization of maximal respiratory responses in the dog similar to that in the cat in the few ex-

periments in which higher stimulus intensities were employed, is unexplained. That it is due to a species difference between cat and dog is improbable in the light of the results of Beaton and Magoun (1941) in the monkey. Furthermore, the experiments of Nicholson (1936) on the dog are most readily explained on the basis of a morphological dissociation of expiratory and inspiratory centers much as found in the cat. The results of Gesell, Bricker and Magee (1936), though interesting, scarcely bear on the question of differentiation between inspiratory and expiratory motor centers. The potentials which they recorded from such afferent structures as the posterior columns, gracile and cuneate nuclei, internal arcuate fibers, tractus solitarius, lateral reticular nucleus, etc., obviously have their origin within proprioceptive end organs responsive to the respiratory movements of the animal. These structures, distributed widely throughout the medulla, contribute both inspiratory and expiratory potentials, and while they probably play in part on the respiratory motor centers, do not represent in themselves activity of the motor centers. Their wide distribution throughout the medulla would tend to obscure activity of the true motor centers.

CONCLUSIONS

1. Utilizing stimuli of moderate intensity, it is possible to perform an adequate physiological localization within the brainstem by the Horsley-Clarke method of stimulation, agreeing well with known morphology.
2. The functional subdivision of the respiratory center into inspiratory and expiratory portions previously proposed by Pitts, Magoun and Ranson (1939a) is affirmed to have a morphological basis.

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THE EFFECTS OF WATER MOCCASIN VENOM ON DOGS

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The action of the venom of the water moccasin, *Agkistrodon piscivorus* Lacépède, has received little physiological study. The principal work has been that of Mitchell and Reichert (1886), Flexner and Noguchi (1903), Noguchi (1909) and Essex (1932). The earlier workers found certain similarities between the actions of *Agkistrodon* and *Crotalus* venoms and noted the greater neurotoxic activity of the former. In his work on moccasin venom, Essex noted only similarities to the results from *Crotalus* venom as determined by him and his collaborators (Essex and Markowitz, 1930; Taube and Essex, 1937). Hence the neurotoxic action of the *Agkistrodon* venom is in question.

Experimental procedure. Dogs were anesthetized with sodium barbital,[2] 280 mgm. per kilogram of body weight, or sodium pentobarbital, 32 mgm. per kilogram, intravenously. Blood pressure was recorded by mercury manometer from the carotid artery, respiration by modified Marey tambour from the tracheal cannula. In some experiments, the phrenic nerves and one sciatic nerve were exposed. Animals were kept warm. The series of experiments is composed of thirty-one dogs.

The venom used was from the batch described by the author (1940). Venom was weighed out and diluted to 0.1 per cent with Ringer, Tyrode, or physiological saline; fresh solutions were made each time.[3] Dosage is expressed in milligrams of dry venom per kilogram of body weight. Injections were by cannula into the femoral vein and were washed in by burette. Duration of injections was variable.

In experiments in which the phrenic nerves were stimulated, the minimal break shock necessary to produce a visible respiratory effect was used. Similarly a minimal value for the tetanizing current was determined for the central end of a sciatic nerve; this value or a greater one was used in each case.

[1] A part of this work was done in the Departments of Physiology and Pharmacology, the University of Chicago.

[2] First four animals only.

[3] Venom-glycerol-Ringer stock solution (Essex, 1932) was used in the first two experiments.

RESULTS. On the basis of respiratory effects the animals may be divided into three groups: group I is composed of animals dying of immediate and absolute respiratory failure; group II consists of animals dying of respiratory failure but showing some respiratory activity, either terminal gasps or progressive depression, before complete failure; group III is composed of animals in which respiratory failure did not occur. These groups contain thirteen, forty-one, and forty-five per cent of the animals respectively.

Group I (fig. 1, A; fig. 2, no. 33). These animals showed fleeting stimulation, with immediate respiratory failure occurring within one minute of the beginning of the venom injection. There were neither terminal gasps nor response to sciatic stimulation: the failure was absolute. Minimal stimulations of the phrenic nerves produced twitches of the diaphragm.

There was an immediate severe fall in blood pressure within one minute of the beginning of the injection. A slow asphyxial rise then began in three cases; the fourth, which received the largest venom injection (1.25 mgm. per kgm.) showed no such rise. Sciatic stimulation usually lowered blood pressure.

The blood clotted well in two animals, poorly in one; the last was not autopsied. There was no hemorrhagic effect in any of them.

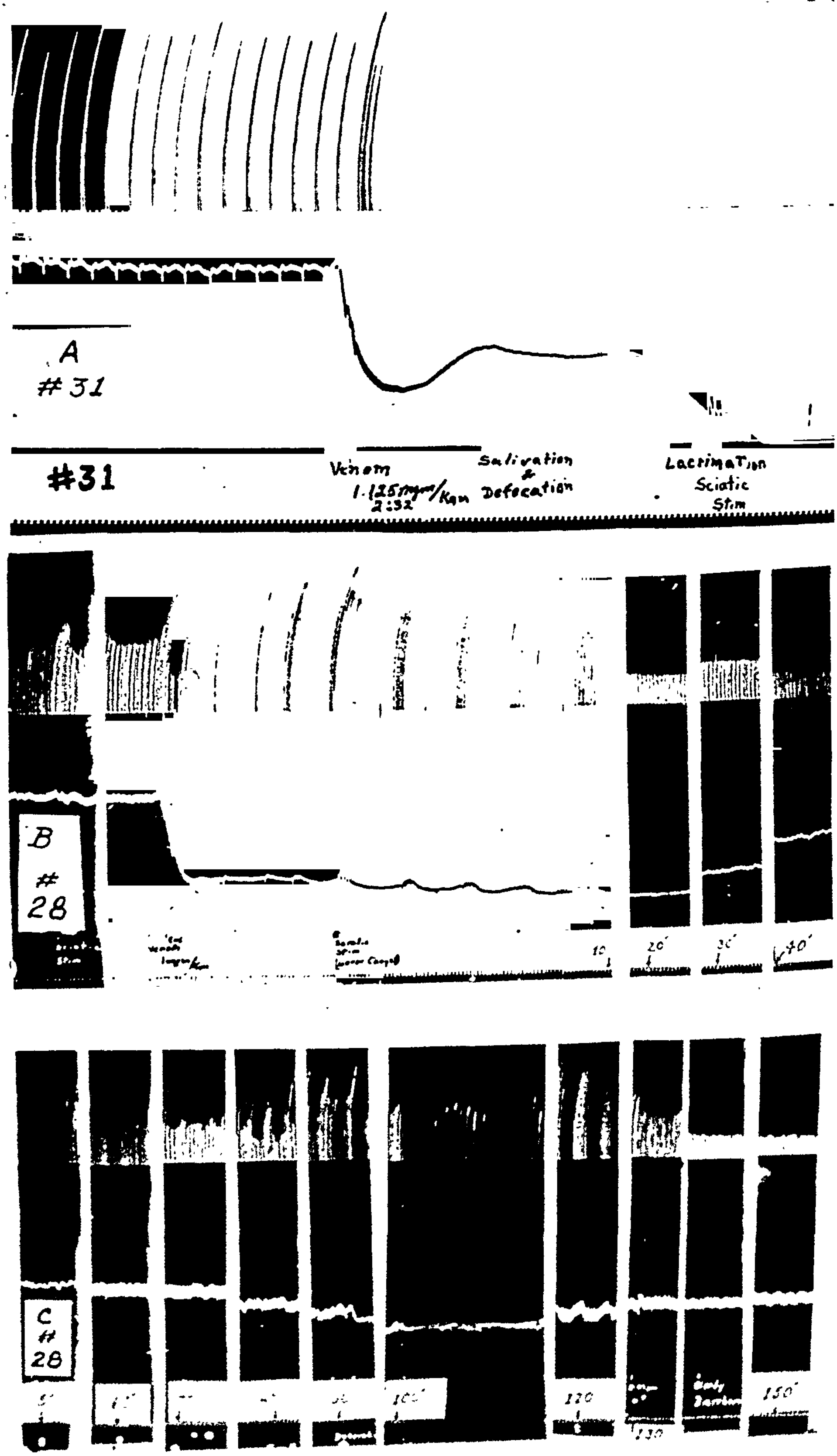
Group II (fig. 2, no. 24 and no. 10). These animals also showed respiratory failure; however, it either developed gradually or terminal gasps were present. Cheyne-Stokes breathing usually occurred in the first event. The effectiveness of sciatic stimulation varied directly with the degree of respiratory activity, being wholly ineffective once failure had occurred. Stimulations of the phrenic nerves produced twitches of the diaphragm after complete respiratory failure.

Blood pressure fell precipitately to forty-three per cent of the original at five minutes after beginning the venom injection. The extent of the asphyxial rise varied inversely with the survival time, being great in animals showing only terminal gasps and almost absent when the respiratory failure appeared very late. Sciatic stimulation usually lowered the blood pressure.

Only two animals were autopsied; one showed congestion; the blood clotted; the other showed occasional hemorrhages and a few clots.

Group III (fig. 1, B; fig. 3). These animals did not show respiratory failure; all received additional venom injections. The results discussed here are those of the first dose only.

The characteristic respiratory sequence after venom was: fleeting stimulation, depression (occasionally complete paralysis for a brief time, usually of the Cheyne-Stokes type), recovery, slight stimulation (approximately 30 per cent above the original), normal respiration. Again there was an inverse relationship between the effectiveness of sciatic stimulation and the degree of respiratory activity.


A
#31
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Venom
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Salivation
&
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FIGURE 1

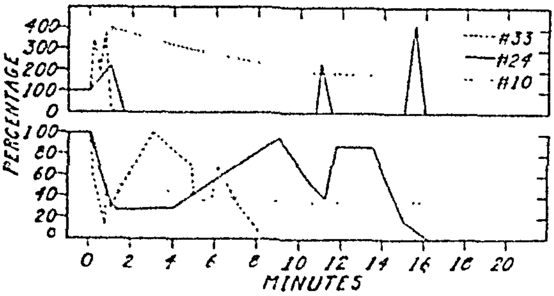

Fig. 2

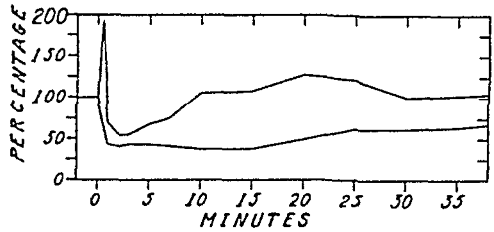

Fig. 3

Fig. 2. Ordinates are in percentages of the normal; abscissae are in minutes after beginning the injection of venom. The upper graph shows respiratory volume, i.e., the product of the rate and amplitude; the lower shows blood pressure.

No. 33 is from group I; note the early respiratory failure and the asphyxial rise in pressure. Venom: 1.125 mgm. per kilogram. (The fall in pressure at five minutes was caused by tetanizing stimulation of the central end of the sciatic nerve.)

Nos. 24 and 10 are from group II; the former shows terminal gasps, the latter early stimulation and gradual failure. Note the corresponding pressure changes. Venom: 1.0 and 0.5 mgm. per kilogram, respectively.

Fig. 3. Ordinates are in percentage of the normal; abscissae are in minutes after beginning of the venom injection. The upper line shows the respiratory effects, the lower the blood pressure effects in animals of group III. The respiration is the average for eleven animals; three showed pure stimulation and are excluded from the average. The blood pressure is the average for all the animals in the group.

TABLE 1

Miscellaneous data for the several groups

All numbers not otherwise designated are in percentage of the group. No average duration is shown for group III, since several injections were given each animal later; the effects of these are not shown here.

GROUP	PERCENTAGE OF TOTAL	SALIVATION	MICTURITION	DEFECATION	BRADYCARDIA	AVERAGE DURATION	PER CENT FALL IN BLOOD PRESSURE	AVERAGE DOSE
						minutes		*mgm./kgm.*
I	13	50	50	25	50	6.5	64	1.13
II	42	41	27	0	59	36.2	57	0.72
III	45	29	7	43	50	—	60	0.67

Circulatory changes correspond with those described by Essex (1932) for moccasin venom and, by inference, rattlesnake venom (Essex and Markowitz, 1930). The description by Taube and Essex (1937) of the

Fig. 1 A. Kymograph record of no. 31. Top line: respiration; second line: blood pressure; third line: zero pressure and record of events; fourth: time in five-second intervals. Anesthetic: sodium pentobarbital, 32 mgm. per kilogram. Venom dose: 1.125 mgm. per kilogram, so marked.

B and C. Kymograph record of no. 28. Lines are the same as in the tracing above. Minutes are marked on all sections except that for 140 minutes; the speed of the paper was changed at 100.25 minutes to show a group of respirations. Defecation occurred at four, ninety, and one hundred forty minutes; the last was mucous and bloody from intense gastro-intestinal hemorrhages.

gross pathology following the injection of rattlesnake venom is essentially applicable to the changes produced by moccasin venom in this group. Especially noteworthy were the absence of gross change in the lungs and the severe hemorrhagic destruction of the pancreas. Animals were only relatively refractory to additional venom injections.

Various data from the several groups are shown in the table.

Discussion. Taube and Essex (1937) and Essex and Markowitz (1930) showed that death from rattlesnake venom was caused by circulatory manifestations, i.e., "crotalin shock." Essex (1932) stated that *by the tests he employed*, "There is not a distinguishable difference in the physiologic action of the venom of the water moccasin and that of the rattlesnake." There is no discussion of respiratory effects either in his paper on moccasin venom or in the extensive series of Essex and his co-workers on rattlesnake venom.

Both the abrupt cessation of respiration in group I and the early or delayed failure in group II, accompanied by an asphyxial rise in blood pressure in both groups indicate acute respiratory paralysis as the cause of death. In view of the transitional nature of group II, the division between group II and III is somewhat arbitrary and is based on the presence of an asphyxial rise in group II which is absent in group III. On this basis 55 per cent of the animals died of respiratory paralysis. Even in group III, 79 per cent of the animals showed severe respiratory depression, with subsequent return to normal respiration. Ninety per cent of the entire series of animals showed severe depression or failure of respiration.

In all the animals in this series of experiments, a precipitate fall in blood pressure occurred; Essex and his co-workers ascribed this fall entirely to peripheral effects. In addition to these peripheral effects, there were central factors. The occurrence of marked asphyxial rises in pressure indicated that the vasomotor center, which had been depressed by the venom, was stimulated by the anoxemia consequent to the low blood pressure, with a concomitant pressure rise. When the asphyxia was delayed, this rise was less, probably because of at least three factors: 1, further venom depression of the vasomotor center; 2, permanent injury of the vasomotor center by the long-continued anoxemia consequent to the persistent low blood pressure; 3, appearance of hemorrhagic and other peripheral effects. When no asphyxia occurred, the survival time was long. Sciatic stimulation produced little vasomotor response, probably for the reasons enumerated above. Attempts to distinguish direct vasomotor depression from anoxemic injury have so far been unsuccessful.

Clotting of blood occurred in animals dying soon after venom injection; where death was delayed, as in group III, the blood remained fluid. Similarly hemorrhagic effects developed only in those animals which lived long enough for vascular destruction to occur (*ca.* 15 min.).

SUMMARY. In this series of dogs, 55 per cent of the animals died of respiratory failure brought about by direct action of the venom on the respiratory center.

Injections of venom caused an immediate *severe fall in blood pressure in* all cases. Animals dying of respiratory failure showed asphyxial rises in all cases but one.

After respiratory failure stimulation of the sciatic nerve produced no respiratory activity. Phrenic nerves were unaffected, as judged by the test employed; i.e., there is no evidence of a curare-like action.

Animals which had received one dose of venom were only relatively refractory to additional doses of venom.

Bradycardia was evident in 52 per cent of the subjects.

Salivation occurred in 38 per cent of the animals; defecation in 19 per cent; lacrimation was present in one case.

Clotting of the blood occurred only in animals dying within a few minutes after the injection; in others the blood remained fluid.

Hemorrhage into the tissues was absent in animals dying within a few minutes of the injection. The extent of hemorrhage varied directly with the duration of life.

CONCLUSION

Poisoning by water moccasin venom is partially due to the neurotoxic action which produces respiratory and vasomotor depression or failure; when these factors do not produce death, the persistent low blood pressure and the hemorrhagic vascular effects are responsible for the mortality. Between these extremes are cases where both sets of factors are active.

There is a definite relationship between the quantity of venom injected and the cause of death: the heavier doses yield the neurotoxic (central) effects and lighter doses the "shock" type (peripheral, or circulatory) effects.

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THE RELATION OF EXTERNAL PANCREATIC SECRETION TO VARIATIONS IN BLOOD SUGAR[1]

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The effects of induced blood-sugar variations on external pancreatic secretion have been studied by numerous investigators with conflicting results. Babkin and Savitsch (1) showed that the intragastric administration of cane sugar and hydrochloric acid, to dogs with permanent pancreatic fistulae, elicited juice of a higher trypsinogen content than could be obtained by the acid alone. La Barre and Destrée (2), employing the cross-circulation method in chloralosed dogs, reported that moderate hypoglycemia of the recipient's encephalic centers diminished the volume and enzyme content of pancreatic juice elicited by intravenous secretin. This inhibitory effect was abolished by bilateral vagotomy. Gayet and Guillaumie (3) using a similar technique observed no increase in pancreatic secretion during hyperglycemia either before or after vagotomy. In anesthetized rabbits, Baxter (4) noted a decrease in the enzyme content of pancreatic juice obtained during insulin hypoglycemia. Vagotomy abolished this response but did not prevent the rise in enzymes evoked by hyperglycemia. Experiments conducted on men by Okada (5) and Frisk and Welin (6) showed the gastric and pancreatic secretion, collected by suction through intraduodenal and intragastric tubes, were augmented by insulin hypoglycemia. The former investigators showed that glucose inhibited this response while the latter obtained a greater rise in enzymes than in volume of pancreatic juice during insulin hypoglycemia.

From the evidence obtained by investigators (for a more complete review see Okada (5), Frisk and Welin (6)) it is evident that the relation of the external pancreatic secretion to blood-sugar variations has not been studied thoroughly in unanesthetized dogs with pancreatic fistulae. In order to obtain clean-cut evidence on this problem and to investigate some of the mechanisms involved, the following experiments were conducted.

METHODS. The majority of the pancreatic fistulae were prepared by the Inlow method. The details for the preparation of this fistula are given

[1] Portions of this paper were presented at the meetings of the American Physiological Society in March, 1940 and April, 1941.

elsewhere (7). In this fistula the transplanted major pancreatic duct permits easy cannulation at each experimental period. The animals were gastrostomized by the method of Carlson (8). Following postoperative recovery each animal was trained to lie on a padded table and pancreatic juice was conducted to an automatic drop counter and recorder by a glass cannula cemented into the transplanted duct with collodion. Intragastric pressure was recorded by a water manometer and condom balloon, the latter was introduced into the fundus of the stomach and inflated to a pressure of 5 cm. of water. The balloon was held in the fundus by a $\frac{1}{8}$ inch stiff-rubber tubing which passed through the balloon and extended beyond for several inches. This tube, with its perforated tip, served to drain fluid gastric content.

The other type of pancreatic fistula used in some of the experiments was prepared by introducing the special gastric and duodenal cannulae of Thomas (9). The duodenal cannula was placed in the intestine directly opposite the major pancreatic duct which permitted direct cannulation of the duct through the duodenal cannula (10). The special gastric cannula was placed in the fundus of the stomach which permitted the introduction of the balloon and drainage tube.

Observations were conducted for 3 to 6 hours on the trained animals which had fasted 18 to 24 hours. One unit of insulin per kilogram (Iletin or crystalline insulin) was injected subcutaneously or intravenously. Glucose (1 gram per kgm.) was injected intravenously, usually as a 20 per cent solution. Blood samples were drawn at appropriate intervals from the saphenous or cephalic vein and the blood sugar was estimated by the Shaffer-Hartmann method on cadmium sulphate filtrates.

Results. *Effects of subcutaneous insulin and intravenous glucose on the volume and proteolytic activity of pancreatic juice.* This group of experiments was conducted on 5 Inlow fistulae which were selected because they secreted large quantities of pancreatic juice and did not show "asecretory" periods (for a discussion of this problem see (11)). During experimental periods the pancreatic juice was collected at half-hour intervals and its proteolytic activity, after the addition of enterokinase, was estimated by formol titration. After a control period of 1 to 2 hours either insulin or glucose, or insulin followed in 50 to 60 minutes by glucose, was injected.

Figures 1, 2 and 3 are graphs of three results obtained. Figure 1 illustrates an experiment in which a reduction in the secretory rate and an augmentation of the proteolytic activity followed the administration of insulin. In the experiment graphed in figure 2 there was little difference in the volume of juice secreted following the insulin but again an increase in the proteolytic activity was observed. Figure 3 shows an increase in the volume and a decrease in the proteolytic activity of the juice collected after the administration of glucose. The data from these experiments are

summarized in table 1. In this table the average volume and the average proteolytic activity of the juice obtained after glucose or insulin administration is compared with the average values obtained during the corresponding control period. The result was recorded as an "increase," "decrease," or "no change."

These experiments demonstrated that no consistent, predictable results could be obtained with insulin or glucose under the above conditions. Babkin (12) has noted that in general the enzyme content of pancreatic

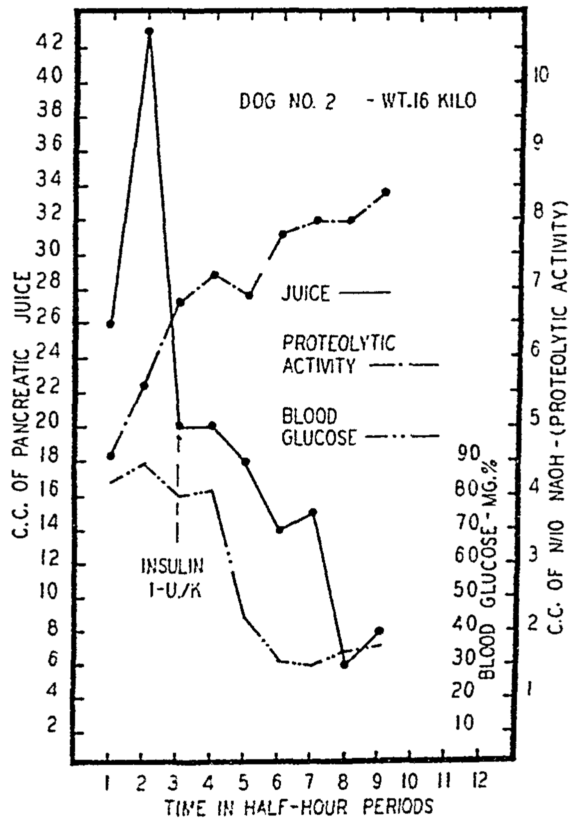

Fig. 1. Experiment showing a reduction in the volume and an increase in the proteolytic activity of pancreatic juice following subcutaneous insulin (1 unit per kgm.).

juice varies inversely with the volume of pancreatic juice. This observation was often confirmed by us in control experiments in which no insulin or glucose was injected. Since after insulin administration the proteolytic activity increased in 8 experiments it is possible that the method used above obscured small increases in this activity.

Effects of insulin and glucose on the volume of pancreatic secretion and on gastric motility. Numerous investigators (13, 14, 15, 16), have reported that insulin hypoglycemia augmented gastric motility; this hypermotility was usually inhibited by intravenous glucose. Since we (17) had observed

a temporal correlation between hunger contractions and pancreatic secretion it seemed possible that insulin might elicit an increase in the volume of pancreatic secretion concomitant with the onset of hypoglycemic gastric hypermotility.

Tables 2, 3 and 4 (group A) summarize the data obtained on 8 dogs employing the procedure outlined under Methods. These data show clearly that hypoglycemia at 50 to 60 minutes following subcutaneous or intravenous insulin was associated with an augmentation of both the gastric motility and the volume of pancreatic juice. This augmentation usually

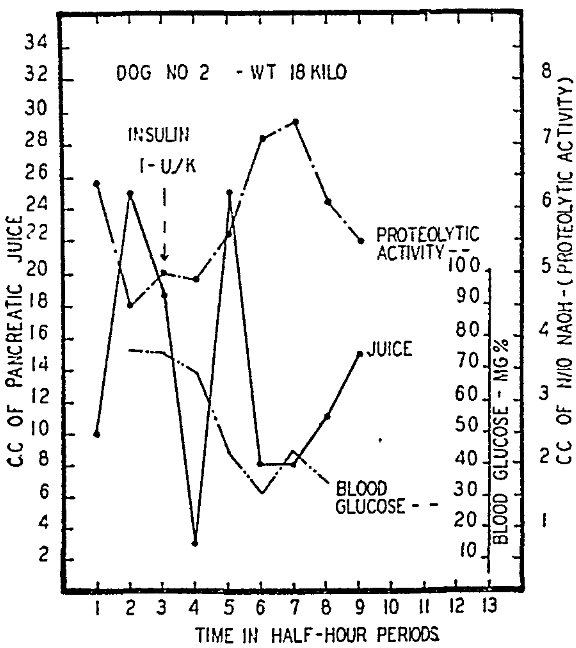

Fig. 2

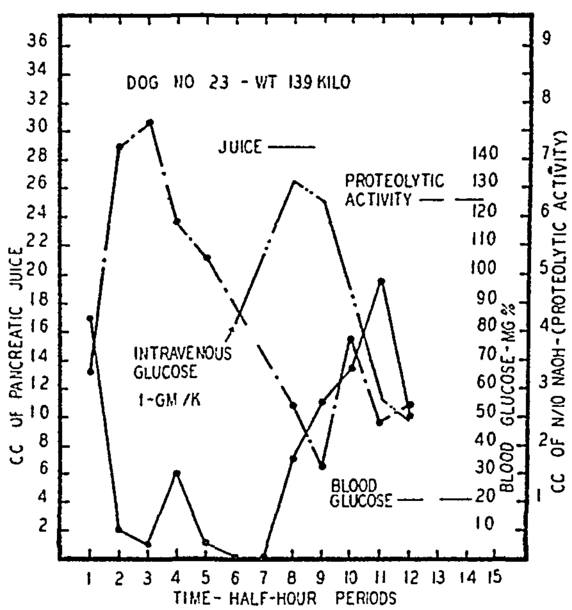

Fig. 3

Fig. 2. Results of an experiment in which no appreciable change in the volume was accompanied by an increase in the proteolytic activity of pancreatic juice after the administration of subcutaneous insulin (1 unit per kgm.).

Fig. 3. Experiment demonstrating an increase in the volume and a decrease in the proteolytic activity of pancreatic juice following intravenous glucose (1 gram per kgm.).

began when the blood sugar reached 40 or 50 mgm. per cent as is shown in figure 4. Usually, but not invariably, the gastric hypermotility preceded the pancreatic response by an interval of several minutes. Typically the hypermotility showed an incomplete tetany together with increased amplitude of gastric contractions. Glucose administered intravenously during the hypoglycemia produced an immediate reduction in the gastric and pancreatic activity (see fig. 4). In a few cases the pancreatic secretion was inhibited completely for 5 to 10 minutes. More often, however, the secretory rate was reduced and the gastric tone and contraction-amplitude was

diminished only to be followed in a short time by a return to a more rapid secretory rate and vigorous gastric contractions. This increase in pancreatic flow often occurred within 15 minutes after the injection of glucose and at a time when the blood sugar was still high. Injection of a hypertonic sodium chloride solution equal to the volume of the glucose solution failed to produce such inhibition and often produced an augmentation (see fig. 4). When a post-glucose hypoglycemia appeared, following the intravenous injection of glucose, the gastric and pancreatic responses were identical with those obtained during insulin hypoglycemia.

TABLE 1

Effect of subcutaneous insulin (1 unit per kgm.) and intravenous glucose (1 gram per kgm.) on volume and proteolytic activity of pancreatic juice

	SECRETION			ENZYME		
	Increase	Decrease	No change	Increase	Decrease	No change
Glucose, 16 experiments.	4	10	2	4	3	9
Insulin, 16 experiments ..	8	5	3	8	3	5

TABLE 2

*Number of experiments showing the effect of 1 unit of insulin per kilogram subcutaneously at 50 to 60 minutes after injection on:**

GROUP	GASTRIC MOTILITY				PANCREATIC SECRETION			
	Augmentation			Inhibition	Augmentation			Inhibition
	Marked effect	Moderate effect	No effect		Marked effect	Moderate effect	No effect	
A	9	7	1	0	10	2	4	0
B	0	0	3	0	2	1	0	0

* Blood glucose 25 to 40 mgm. per cent.

Group A: Experiments on dogs before bilateral vagotomy.

Group B: Experiments on dogs after sectioning left vagus in the neck and right vagus in the thorax.

Regan (14) reported that an immediate transitory inhibition of gastric motility followed by the typical hypoglycemic augmentation appeared when intravenous insulin was administered. This reaction was observed in our experiments along with a concomitant inhibition of pancreatic secretion when either crystalline insulin or Iletin was used. While the initial pancreatic inhibition was sometimes complete, more often the secretory rate was merely diminished.

Effects of insulin and glucose on the volume of pancreatic secretion and on the gastric motility after bilateral vagotomy. In order to study the effects of

TABLE 3

Number of experiments showing the effect of intravenous injection of 1 unit of insulin per kilogram

GROUP	IMMEDIATE EFFECT ON:							
	Gastric Motility				Pancreatic Secretion			
	Inhibition			Augmentation	Inhibition			Augmentation
	Marked effect	Moderate effect	Little or no effect		Marked effect	Moderate effect	Little or no effect	
A	8	17	4	0	11	13	4	0
B	0	8	2	0	2	4	0	0
C	4	3	9	0	1	3	12	2
	EFFECT AT 50 TO 60 MINUTES FOLLOWING INJECTION ON:*							
	Gastric Motility				Pancreatic Secretion			
	Augmentation			Inhibition	Augmentation			Inhibition
	Marked effect	Moderate effect	Little or no effect		Marked effect	Moderate effect	Little or no effect	
A	17	5	2	0	17	7	5	0
B	0	0	9	0	5	4	0	0
C	0	0	17	0	0	0	17†	0

* Blood glucose 25 to 40 mgm. per cent.

Group A: Experiments on dogs before bilateral vagotomy.

Group B: Experiments on dogs after sectioning left vagus in the neck and right vagus in the thorax.

Group C: Experiments on dogs after *complete* vagotomy.

† Note: six of these showed a very *slight* augmentation.

TABLE 4

*Number of experiments showing the effect of 1 gram of glucose per kilogram intravenously on:**

GROUP	GASTRIC MOTILITY				PANCREATIC SECRETION			
	Inhibition			Augmentation	Inhibition			Augmentation
	Marked effect	Moderate effect	Little or no effect		Marked effect	Moderate effect	Little or no effect	
A	15	23	8	0	28	14	2	1
B	0	1	17	1	12	5	0	0
C	0	0	17	1	6	8	3	1

* Blood glucose 200 to 300 mgm. per cent.

Group A: Experiments on dogs before bilateral vagotomy.

Group B: Experiments on dogs after sectioning left vagus in the neck and right vagus in the thorax.

Group C: Experiments on dogs after *complete* vagotomy.

bilateral vagotomy on the gastric motility and pancreatic responses to insulin and glucose the following two-stage operations were performed and

the animals were studied, after recovery, under the conditions previously described. Two groups of animals were prepared: group B consisted of 4 incompletely vagotomized dogs (the right vagus was cut in the mid-thoracic region, the left was cut in the neck); group C was composed of 3 completely vagotomized dogs, two dogs having bilateral cervical vagotomies while the other had both vagi sectioned just above the diaphragm after a left cervical vagotomy.

The results of experiments conducted on the group B animals (see tables 3 and 4) showed that the immediate inhibitory effect of intravenous insulin on the gastric motility and pancreatic secretion usually persisted. The hypoglycemic effects, however, were markedly altered by the incomplete vagotomies since the rapid pancreatic secretory responses appeared but not the gastric hypermotility. The stomachs of these animals remained atonic

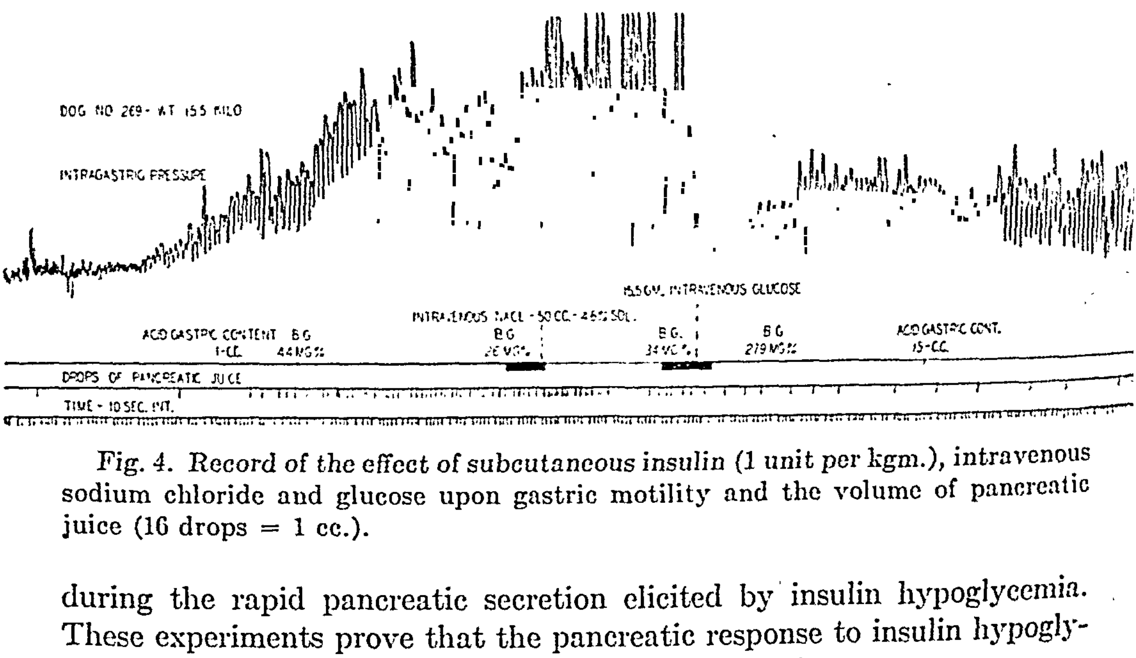

Fig. 4. Record of the effect of subcutaneous insulin (1 unit per kgm.), intravenous sodium chloride and glucose upon gastric motility and the volume of pancreatic juice (16 drops = 1 cc.).

during the rapid pancreatic secretion elicited by insulin hypoglycemia. These experiments prove that the pancreatic response to insulin hypoglycemia can be independent of the gastric motor activity.

In the completely vagotomized dogs (group C) the gastric motor response and the rapid pancreatic secretion of insulin hypoglycemia were both abolished (see group C, tables 3 and 4). In no experiment of this group was more than a very slight pancreatic augmentation elicited by insulin hypoglycemia. The initial inhibitory action of intravenous insulin, which was so frequently observed in the other experiments, was usually absent.

In both groups of animals (B and C) intravenous glucose produced no significant change in the atonic hypoglycemic stomach but almost always elicited a transient, marked or moderate reduction in the pancreatic secretory rate.

These experiments show that complete vagotomy abolishes the normal

motility and secretory response to insulin hypoglycemia but does not prevent an inhibitory action of intravenous glucose upon the pancreatic secretion. Complete vagotomy appears also to reduce the incidence of the inhibitory responses of the pancreas immediately following the intravenous injection of insulin.

The effects of excluding gastric juice from the duodenum upon the pancreatic secretory response to insulin hypoglycemia. Babkin (18) reported that insulin elicited a large flow of gastric juice which resulted from the hypoglycemic stimulation of the vagus centers because the gastric response was abolished by vagotomy. In view of this mechanism it seemed possible that the pancreatic response observed by us could have resulted from the entrance of gastric acid into the duodenum with the formation of secretin which called forth a rapid pancreatic secretion. In order to determine whether this mechanism played a part in the hypoglycemic pancreatic response the following experiments were performed. Because vagotomy can alter the gastric and pancreatic responses to insulin it was necessary to exclude gastric juice from the duodenum without extensive damage to the nerve supply of the stomach or pancreas. To avoid such damage the 3 dogs with gastric and pancreatic fistulae were subjected to sub-muscular "pyloric separations". In this operation the thick muscular layers of the stomach were incised longitudinally and completely separated from the underlying submucosa at a level just rostral to the pyloric sphincter. The gastric mucosa and submucosa were divided and the cut ends were so closed as to completely separate the lumen of the stomach from the duodenum. The longitudinal incision was carefully sutured to avoid extensive destruction of the nerves which are present in this region.

In 6 experiments conducted on these animals insulin produced a typical gastric hypermotility during hypoglycemia but failed to augment the rate of pancreatic secretion significantly. In no instance was the rapid pancreatic secretion observed which was the characteristic response of these animals prior to pyloric separation. These experiments were conducted on the animals after healing of the abdominal wounds had occurred. In a number of semi-acute experiments insulin failed to initiate either gastric hypermotility or pancreatic augmentation. These experiments demonstrate that closure of the stomach at the pylorus prevents the rapid secretory response of the pancreas normally seen during insulin hypoglycemia.

The relation of "spontaneous" blood-sugar variations to gastric motility and pancreatic secretion. E. B. Boldyreff (19) reported that during the "work" periods of the stomach and pancreas the blood sugar fell 20 to 30 mgm. per cent and returned to higher levels during the "rest" periods of these organs. Mulinos (16) (on dogs) and W. W. Scott (20) (on men) failed to establish any causal relation between the blood-sugar level and gastric hunger motility. In order to extend these observations to include fasting

pancreatic secretion 30 experiments on 7 dogs were performed; in 11 of these experiments the pancreatic secretion was led to the exterior and recorded; in the remainder of the experiments the pancreatic juice entered the duodenum naturally and only the motility of the stomach was recorded. Table 5 shows a typical experiment in which no significant changes in blood sugar occurred during marked gastric and pancreatic activity. It will be noted that the pancreatic flow ceased during the intermotility period and that the blood sugar was higher during the subsequent period of hunger contractions than during the period of quiescence of the stomach and pancreas. The experiments in which the pancreatic juice was not diverted from the intestine also failed to show the positive correlation between the blood sugar and the gastric motility claimed by Boldyreff.

TABLE 5

Blood sugar, gastric motility and pancreatic secretion

TIME	BLOOD SUGAR	MAXIMUM INTRAGASTRIC PRESSURE	DROPS OF PANCREATIC JUICE PER MINUTE
	milligrams per cent	*centimeters of water*	
8:15 a.m.	66	27	7
9:01 a.m.	65	29	12
9:33 a.m.	76	33	10
10:13 a.m.	76	6*	0
10:48 a.m.	80	15	2
11:10 a.m.	75	23	11

* Stomach showed no hunger motility during this period.

Discussion. It is evident from the foregoing observations that insulin produces a rapid flow of pancreatic juice, together with gastric hypermotility, when the blood sugar is sufficiently reduced. The consistent appearance of the gastric motility response to hypoglycemia confirms the observations of previous investigators (13, 14, 15, 16). In our initial experiments the use of dogs which were "hypersecreting" (Babkin, 12) probably accounts for our original failure to demonstrate the stimulating effects of insulin hypoglycemia on the external pancreatic secretion.

The effects of vagotomy on the volume of pancreatic juice secreted appear to depend upon the completeness of the vagotomy. When both vagi are sectioned in the neck the motility of the stomach and the increased pancreatic secretion seen in insulin hypoglycemia are lost. On the other hand, incomplete vagotomy does not abolish the pancreatic response but only the typical gastric hypermotility of hypoglycemia. The occasional very slight increases in the pancreatic secretion, seen during hypoglycemia in the completely vagotomized dog, were always so small as to be questionable increases. Contrary to Boldyreff (21) we found that insulin

elicited a hypoglycemia after complete vagotomy. Our results confirm, in general, the observations of Okada (5) made on human subjects. These results were less precise than ours because the aspirated pancreatic juice was contaminated with bile and succus entericus.

The immediate inhibitory action of intravenous insulin on gastric motility and pancreatic secretion is most marked and most frequent in the animals before vagotomy. Even incomplete vagotomy often reduces the magnitude of this inhibition. In a few experiments on completely vagotomized dogs the pancreatic flow accelerated immediately after the intravenous injection of insulin. It is possible that intravenous insulin stimulates the centers which control the vagus constrictor fibers to the pancreatic ducts. Special experiments designed to investigate this problem will be needed to settle this question.

The transient inhibitory action of intravenous glucose on the pancreatic secretion persists after complete vagotomy. It seems probable that this inhibitory effect of glucose is exerted directly on the pancreas because this effect persisted in several splanchnicotomized dogs. In 6 experiments intravenous glucose inhibited the spontaneous pancreatic secretion, the inhibition lasting for as long as 20 minutes but usually for a shorter period. The effect resembled the transient inhibitory effect of glucose seen during hypoglycemia.

In considering the way in which the pancreas is excited to secrete rapidly during hypoglycemia it is necessary to consider the secretin mechanism which could play an important rôle in this response. Complete vagotomy abolishes the typical hypoglycemic pancreatic response and also abolishes the typical gastric secretion under these conditions. These results could be explained on the removal of the secretin mechanism. In animals with pyloric separations no gastric juice can enter the duodenum; these animals react to insulin hypoglycemia exactly like the completely vagotomized dogs. This is strong evidence for the dependence of the pancreatic response upon the gastric secretory response. The simplest explanation of these results is that in both conditions the normal secretin mechanism has been eliminated. Frisk and Welin (6) failed to observe marked pancreatic responses in men during insulin hypoglycemia, when the gastric content was continuously aspirated by the gastric tube. Okada (5), however, has observed hypoglycemic pancreatic augmentation in cases of gastric carcinoma which secreted alkaline gastric juice. These results are difficult to reconcile with our observations. Additional experiments, designed to detect the liberation of secretin under the above conditions, are needed definitely to settle this problem.

The authors are indebted to Dr. F. B. Peck, Lilly Research Laboratories, and to Dr. H. Jensen, Squibb Institute for Medical Research, for the Iletin and crystalline insulin used in these experiments.

CONCLUSIONS

1. Insulin hypoglycemia increases the volume of pancreatic juice secreted by unanesthetized dogs having permanent pancreatic fistulae.
2. Intravenous glucose temporarily inhibits the pancreatic secretion which appears spontaneously or in response to insulin.
3. Complete bilateral vagotomy abolishes the rapid flow of pancreatic juice and the gastric hypermotility of insulin hypoglycemia; incomplete vagotomy abolishes the gastric but not the pancreatic response.
4. Intravenous insulin exerts a transient inhibitory effect on the fasting gastric motility and pancreatic secretion; the incidence of this effect is reduced by complete vagotomy.
5. Exclusion of the gastric juice from the duodenum by pyloric separation abolishes the rapid pancreatic secretion of insulin hypoglycemia.
6. Spontaneous variations in the volume of the fasting pancreatic secretion and gastric motility are unrelated to the fluctuations in blood sugar.

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THE PRODUCTION OF EXPERIMENTAL POLYCYTHEMIA IN DOGS, RABBITS AND MAN BY THE DAILY ADMINISTRATION OF EPHEDRINE; AND BY AMPHETAMINE IN DOGS

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We have shown previously (1) that choline and other vasodilator drugs are effective in depressing the experimental polycythemias produced in dogs by cobalt administration or by daily exposure to low atmospheric pressure. To explain this action of the above drugs, we assume that they improve the blood flow to bone marrow, thus diminishing the local anoxia and removing the stimulus to polycythemia.

If this explanation is correct, it would seem that drugs which might constrict marrow arterioles should have the opposite effect; i.e., should increase the rate of red blood cell formation.

The present investigation was made, therefore, to see whether a polycythemia could be produced by certain drugs which have the general action of producing vasoconstriction. Ephedrine and amphetamine (benzedrine) were chosen for this trial because of their prolonged action and resistance to destruction by the body.

Procedure. The subjects used for these experiments consisted of 6 dogs, 7 rabbits and one human. The dogs and rabbits were fed a constant adequate diet and were allowed water ad libitum.

Control observations on the blood were made over a period of at least two weeks before the drug administrations were commenced. These observations included erythrocyte counts, hemoglobin determinations (Sahli), total leukocyte counts, and estimation of the percentage of reticulocytes. The latter observation was made using glass slides on which a drop of blood had been mixed and smeared with a drop of aqueous cresyl blue solution, dried, and then stained with Wright's stain.

Blood samples were drawn from the saphenous vein in the dogs, and only at times when the animals were in an unexcited and basal state. In the rabbits blood was drawn directly into diluting pipettes from the site of puncture of the marginal ear vein. During the experimental periods, blood was sampled only after the elapse of at least 17 hours from the time at which the daily dose of drug had been given.

Ephedrine sulfate was administered to four dogs by stomach tube in daily doses ranging from 2.5 to 5.0 mgm. per kilogram. The latter dose level has been shown by Ogden and Teather (2) to raise the blood pressure in dogs following oral or subcutaneous administration.

In similar experiments on rabbits, 2 normal and 2 splenectomized rabbits were given 45 mgm. of ephedrine sulfate daily by subcutaneous injection.

Amphetamine (Benzedrine) sulfate was given orally to one splenectomized and 3 normal dogs in a daily dose of 10 mgm. The same daily dose was administered by stomach tube to 4 rabbits, 2 of which were splenectomized.

Three rabbits with cobalt polycythemia were given daily injections of 45 mgm. of ephedrine sulfate in addition to daily injections of 10 mgm. of

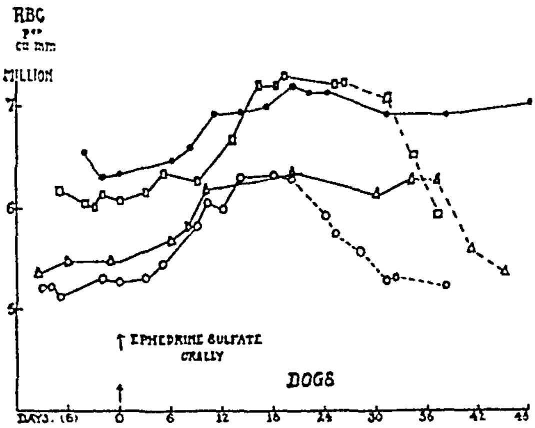

Fig. 1

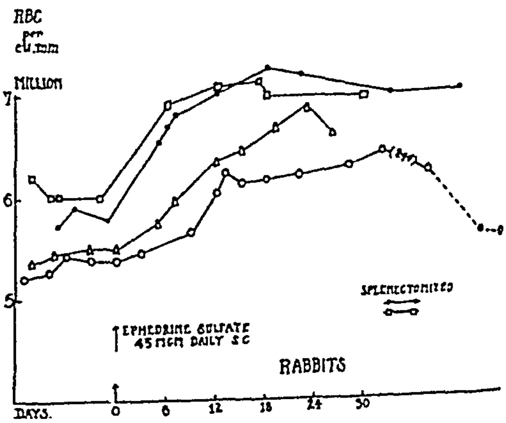

Fig. 2

Fig. 1. The production of polycythemia in dogs by the daily administration of 2.5 to 5.0 mgm. of ephedrine sulfate per kilogram by stomach tube. Dashes indicate cessation of ephedrine feeding.

Fig. 2. The effect of daily subcutaneous injection of 45 mgm. of ephedrine sulfate into 2 normal and 2 splenectomized rabbits, upon their red blood cell counts.

cobalt (40 mgm. cobalt chloride) which was the dose used to induce this type of polycythemia.

One human subject ingested ephedrine sulfate in a daily dose of 50 mgm. for 20 days.

Results. The daily oral administration of ephedrine to 4 normal dogs caused an increase of about one million in their basal erythrocyte numbers (fig. 1). The development of polycythemia was gradual. No significant increase of red cells occurred during the first 6 days. Within 10 to 15 days, however, the full increases in red cell numbers were apparent. They persisted throughout the periods of ephedrine administration and dropped back to normal within 7 to 10 days after the discontinuation of ephedrine. The cessation of the drug feeding in each dog is indicated by the beginning of the dashes in figure 1. Hemoglobin percentages (not shown) ran parallel

with the red cell counts, throughout. The reticulocyte percentages were approximately doubled during the polycythemia.

Total leukocyte counts remained approximately constant for each dog and did not vary by more than 10 per cent throughout the experiments. The counts are not shown here, but the normal values ranged from 13000 to 15000 per cu. mm. of blood.

Figure 2 shows the effect of daily injections of 45 mgm. of ephedrine sulfate upon the erythrocyte numbers of 2 normal and 2 splenectomized rabbits. Within one to two weeks the red cell counts of all 4 rabbits had increased by about one million. Reticulocyte percentages (not shown) were also increased from a normal value of 1.5 (average) to 4.0 during polycythemia.

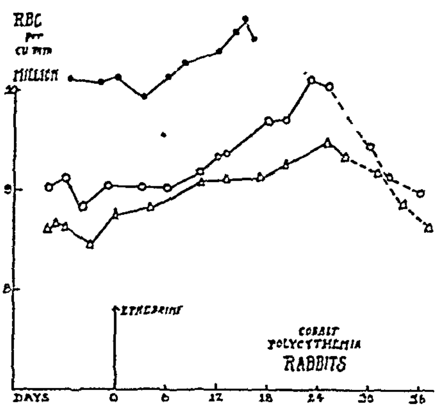

Fig. 3

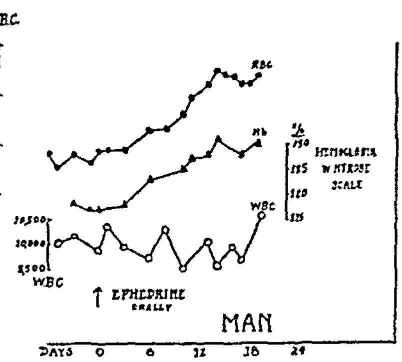

Fig. 4

Fig. 3. The additive effect of daily injections of ephedrine (45 mgm., subcutaneously) upon the blood of rabbits with cobalt-induced polycythemia.

Fig. 4. The effect of daily ingestion of 50 mgm. of ephedrine sulfate upon the blood of one human subject.

Ephedrine sulfate (45 mgm. daily) was injected into three rabbits which already had cobalt polycythemia (fig. 3). This produced further increases in the red cell counts, which amounted to about one half to one million cells per cubic millimeter in the different animals. The cobalt injected rabbits received 10 mgm. of cobalt daily in the form of the chloride; and at the time at which ephedrine injections were started, each rabbit had a polycythemia of about 50 per cent, which had required about two and a half months to develop.

One human subject (the author) took ephedrine sulfate by mouth in a daily dose of 50 mgm., with results on the blood which are shown in figure 4. An increase of about 0.8 million in the erythrocyte number was produced in two weeks. This was accompanied by an increase in hemoglobin of about 11 per cent. Total leukocyte counts, however, did not go up but remained fairly constant around a value of ten thousand. In this experi-

ment it was observed that the first few daily doses of ephedrine raised systolic blood pressure 15 to 20 mm., diastolic pressure about 5 mm., and slowed the pulse rate by about 10 beats per minute. These changes commenced about 40 minutes after ingestion of the drug and persisted for at least 2 hours. Subsequent daily doses of ephedrine did not appear to alter the systolic pressure very markedly. No unpleasant symptoms were experienced as a result of taking the drug. An E.C.G. taken one hour and fifty minutes after the 20th daily dose of ephedrine appeared to be quite normal.

Amphetamine sulfate was given orally to one splenectomized and 3 normal dogs in a daily dose of 10 mgm. As can be seen in figure 5, all four dogs developed polycythemia within 12 days. Erythrocyte counts increased by about one million while hemoglobin percentages (not shown)

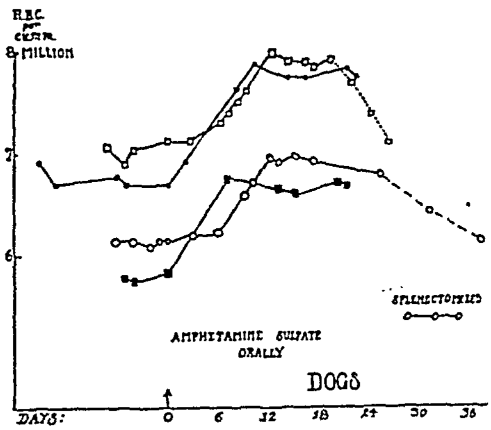

Fig. 5. The development of experimental polycythemia in dogs by the daily oral administration of 10 mgm. of amphetamine sulfate. Dashes indicate cessation of drug feeding.

increased, but not so greatly. Recovery from polycythemia is not shown for 3 dogs in figure 5, since it was desired to do other experiments on these animals. In the one animal in which recovery is illustrated (fig. 5), about 12 days were required for this to occur.

Amphetamine sulfate (10 mgm. daily) was also fed to 4 rabbits by stomach tube. Two of these rabbits apparently developed a polycythemia, but the other two did not, although they received the drug for about eighteen days. Since the results were inconclusive, they are not shown in this paper.

Discussion. The slow onset of polycythemia in dogs, rabbits and man following the commencement of daily ephedrine administration as well as its slow regression upon cessation of the drug indicates a probable true increase in erythropoiesis. This interpretation is also supported by the reticulocytosis observed in dogs and rabbits during these experiments.

The fact that splenectomized animals responded in the same way as normals to ephedrine or amphetamine also supports our belief that these drugs stimulate red blood cell production.

The remote possibility that ephedrine might cause a slow chronic change in state of blood reservoirs and thus increase the erythrocyte number, is largely precluded by the appearance of polycythemia in our splenectomized animals (figs. 2 and 5). Furthermore, the induction of polycythemia in man by ephedrine (fig. 4) does not seem explainable on this basis when viewed in the light of recent work by Ebert and Stead (3). These authors present evidence tending to show that the erythrocytosis observed in normal men as an acute response to exercise or epinephrine injection is not due to contraction of blood reservoirs. Their evidence even casts doubt upon the existence of blood (storage) reservoirs in the human organism.

Dehydration of the blood probably plays no part in the polycythemias reported in this paper. In the first place, fluid loss by the blood might be expected to occur after the first daily dose of drug, if at all; but no significant increase of basal erythrocyte numbers occurred in any of our animals during the first week (figs. 1–5). Secondly, the relative constancy of normal leukocyte counts in man (fig. 4) and dogs (not shown) during ephedrine polycythemia, seems to argue against a possible blood concentration.

The additive effect produced by ephedrine upon rabbits with cobalt-induced polycythemia (fig. 3) would appear to show that this drug stimulates erythropoiesis by a different mechanism than the metal. A theory on the mechanism of cobalt polycythemia has previously been advanced by the author (4) who found that it differed from the polycythemia induced by exposure to low atmospheric pressure, in certain respects. The cobalt polycythemias in rabbits (fig. 3) which represent increases of approximately 50 per cent over the normal erythrocyte numbers, are incidentally greater in extent than any which we have found reported for this species in the literature.

As to the mechanism by which ephedrine stimulates bone marrow, the most plausible explanation seems to reside in a reduction of blood flow to this tissue through vasoconstriction, with a consequent diminution of its oxygen supply. We have previously shown (1) that choline and its derivatives may have the opposite effect, i.e., they depress excess erythropoiesis in polycythemic dogs, probably by improving the blood supply to bone marrow, since their action is blocked by atropine.

Local hypoxia of bone marrow as the stimulus to polycythemia by ephedrine seems plausible from the standpoint of the *time required* to induce the full rise in the erythrocyte number, which agrees generally with the time required to induce polycythemia in dogs by exposure to low atmospheric pressure (5). It may be assumed, perhaps, that the resultant

immediate stimulus to polycythemia is the same in either case (i.e.) hypoxia of marrow.

According to Warren (6) bone marrow metabolism is not under nervous control in rabbits exposed to low atmospheric pressure. We may assume that ephedrine does not act through the nervous system, but rather directly on marrow arterioles.

The mechanism of the production of polycythemia by benzedrine (amphetamine) in dogs is probably the same as that of ephedrine, since the responses of the red cell counts are the same for each drug.

SUMMARY AND CONCLUSIONS

The daily oral administration of ephedrine sulfate to 4 normal dogs, in doses ranging from 2.5 to 5 mgm. per kilogram, caused significant increases in the basal erythrocyte numbers of all of the animals within 10 to 15 days. The red cell counts and hemoglobin percentages remained elevated throughout the period of ephedrine feeding (3 to 7 weeks, or more) and returned to their normal values within 7 to 10 days after cessation of drug administration. Total leukocyte counts remained fairly constant.

Two normal and two splenectomized rabbits which received 45 mgm. of ephedrine sulfate daily by subcutaneous injection, developed polycythemia with reticulocytosis, within two weeks.

The daily injection of ephedrine into 3 rabbits which had a marked cobalt-induced polycythemia, produced further appreciable increases in their erythrocyte numbers.

A significant polycythemia was produced in one human subject in 2 weeks by the daily ingestion of 50 mgm. of ephedrine sulfate. The total leukocyte count did not increase, or change significantly.

The oral administration of 10 mgm. of amphetamine sulfate daily to one splenectomized and 3 normal dogs caused significant increases in their basal red cell counts and hemoglobin percentages within 12 days.

These results can be explained by assuming that ephedrine and amphetamine reduce the blood flow to bone marrow, thus diminishing its oxygen supply, and thereby stimulating erythropoiesis.

Acknowledgment. The author wishes to express his appreciation to Prof. H. B. Pierce for valuable suggestions made in connection with this work, and to Messrs. Dean Wheeler and John Prybylo for technical assistance.

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CATION DISTRIBUTION IN THE MUSCLES OF ADRENALECTOMIZED RATS[1]

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The changes in both the serum electrolyte pattern and also in the electrolyte balance associated with adrenal insufficiency, either untreated or treated in a variety of ways, have been the subject of intensive study by many investigators. Also certain phases of carbohydrate metabolism have been shown to be under the direct hormonal regulation of the adrenal cortex (1, 2). Appreciating the definite but poorly understood correlation between normal carbohydrate metabolism and changes in the concentration of certain serum electrolytes the authors felt that much might be learned concerning the fundamental nature of adrenal insufficiency and perhaps also of normal carbohydrate metabolism by the analysis of the tissues themselves (muscle and liver) for electrolytes as well as for the intermediary products in the metabolism of carbohydrates. To this end they collected the skeletal muscles and livers of the same rats whose carbohydrate metabolism was reported upon in 1936 and analyzed these tissues for the four common cations of known physiological importance, namely, sodium, potassium, calcium and magnesium. Such significant changes in the cation contents of the muscles (but not in the livers) were found that the authors were unwilling to report the results at that time (1937) without further amplification.

In the meantime, tissue analyses for electrolytes have been reported by other workers. Harrison and Darrow (3) found, as had we, that there were greatly increased amounts of potassium and somewhat decreased amounts of sodium in the skeletal muscles of rats in adrenal insufficiency.

Experimental. In the experiments to be reported in detail the procedure was as follows: Male albino rats of the Sprague Dawley strain were kept for two weeks or more under laboratory conditions on a diet consisting of Purina Dog Chow[2], on which they grew rapidly. The animals were

[1] This study was aided by a grant to Dr. George W. Thorn from the Committee on Research in Endocrinology, National Research Council.

[2] The cation content of this food was reported by Dr. H. J. Smith to be: Mg 0.09 per cent, K 0.56 per cent, Na 0.67 per cent and Ca 1.5 per cent.

young, but were permitted to grow to a weight of 250 to 300 grams in order that there might be enough muscle available to permit the analysis for the four cations in the muscles from the same animal. Bilateral adrenalectomy was performed under nembutal anesthesia by the lumbar route[3]. In one group of animals a pellet weighing 80 mgm. of pure synthetic desoxycorticosterone acetate[4] was inserted subcutaneously at the time of operation. During the period of recovery from the operation all of the rats were given 0.9 per cent sodium chloride solution to drink instead of water. Tap water was then substituted for saline in those animals in which adrenal insufficiency was allowed to develop. After a period of seven days without specific therapy the animals were sacrificed without a period of fasting regardless of the degree of insufficiency which had developed. In no instance was the animal moribund. Each animal was subjected to the same routine. Sodium nembutal was injected intraperitoneally. When anesthesia was complete, the heart was exposed and blood was withdrawn under oil into a syringe[5]. The animal was then skinned and the skeletal muscle was dissected from the bones, care being taken that corresponding muscles were used in all cases. The finely cut-up muscle was introduced into a tared flask, ashing and analysis being carried out as described in a previous paper (4). In these experiments, calcium was precipitated as oxalate at pH 4.2 in a centrifuge tube. The supernatant fluid and washings were collected in a 100 ml. volumetric flask, made up to volume, and appropriate aliquots taken for the determination of magnesium by the estimation of the phosphorus in precipitated magnesium ammonium phosphate. Duplicate determinations were run of potassium and magnesium, but not of sodium and calcium.

Since potassium was found to be the cation which showed the largest quantitative variations in the muscles of the different groups of animals the results have been reported in a series of charts designed to illustrate the simultaneous variations between potassium and each of the other cations in individual animals. A summary of the detailed data, presented graphically in the charts, has been recorded in table 1 as average figures for the indicated number of animals. The points illustrated by the charts and table will be discussed in detail.

Discussion. *Variations in the normal values of muscle potassium.* One unexpected feature of these results was the great variability of the values for muscle potassium in all of the groups studied including the group of normal animals[6]. If the values plotted on the abscissa in figure 1 are

[3] The authors are indebted to Dr. Roger Lewis and to Dr. George Koepf for assistance with the operations and care of the animals.

[4] The synthetic desoxycorticosterone acetate used in this study was kindly supplied by Dr. Ernst Oppenheimer of the Ciba Pharmaceutical Products, Inc.

[5] The blood from animals similarly treated was pooled.

[6] This point would have been missed completely had pooled specimens been analyzed.

disregarded for the moment, the values for potassium may be read on the ordinate. Because of the large range in potassium values a suitable scale was necessarily chosen to permit the presentation of all of the points obtained. In figure 2 the same data have been plotted with the omission of the points representing the animals in adrenal insufficiency. Here the scale representing potassium values permits a ready demonstration of the range of variations found among the normal animals (open triangle).

According to the Conway-Boyle theory (5), the muscle cell is normally freely permeable to the potassium ion. Also the concentration of cellular

TABLE 1

Summary of analysis of skeletal muscles of rats

NUMBER OF ANIMALS	CONDITION OF ANIMALS	SPECIFIC THERAPY	MOIST SKELETAL MUSCLE (GRAMS PER CENT)		DRY, FAT-FREE MUSCLE (MEQ. PER 100 GRAMS)					SERUM				
			Moisture	Lipid	Na	K	Ca	Mg	Total base	Na per liter	K per liter	Ca per liter	Cl per liter	N.P.N. per 100 ml.
										meq.	*meq.*	*meq.*	*meq.*	*mgm.*
11	Normal	None	75.0	1.7	10.0	32.0	1.4	8.3	51.7	144.2	6.6	5.5	98.4	34
4	Normal	Desoxycorticosterone acetate pellets	74.3	2.3	14.1	30.2	1.1	7.8	53.2	142.7	6.2	5.3	88.4	34
11	Adrenalectomized compensated	Sodium chloride	75.2	1.9	9.5	36.1	1.2	7.1	53.9	143.4	7.0	5.2	107.3	48
8	Adrenalectomized compensated	Desoxycorticosterone acetate pellets	75.8	1.3	15.6	29.9	1.4	6.8	53.7	141.4	7.8	4.3	93.4	36
11	Adrenalectomized. In insufficiency	None	76.0	1.1	8.9	118.1	1.4	5.9	134.3	133.7	8.5	5.8	94.0	46

potassium would be expected to vary in different phases of carbohydrate metabolism since "the impermeable non-colloidal anions are mostly phosphorylated compounds important for the carbohydrate cycle. If such compounds decrease in concentration during rapid carbohydrate oxidation, potassium should leave the cell, and when reformed the reverse should occur." It seems reasonable to expect, therefore, that there should be large variations in the muscle potassium of normal unfasted animals in different phases of carbohydrate metabolism. The data suggest that in normal animals the muscle potassium falls into two categories; 1, a constant minimum quantity (about 26 meq. per 100 grams of dry fat-free

muscle), and 2, a variable quantity (which in these experiments was represented by increments amounting sometimes to as much as 20 meq.). The former may represent the potassium which is held within the cells in the basal condition (i.e., "basal potassium"), the latter may represent that which enters the cells to meet the changing temporary demands of carbohydrate metabolism (i.e., "metabolic potassium").

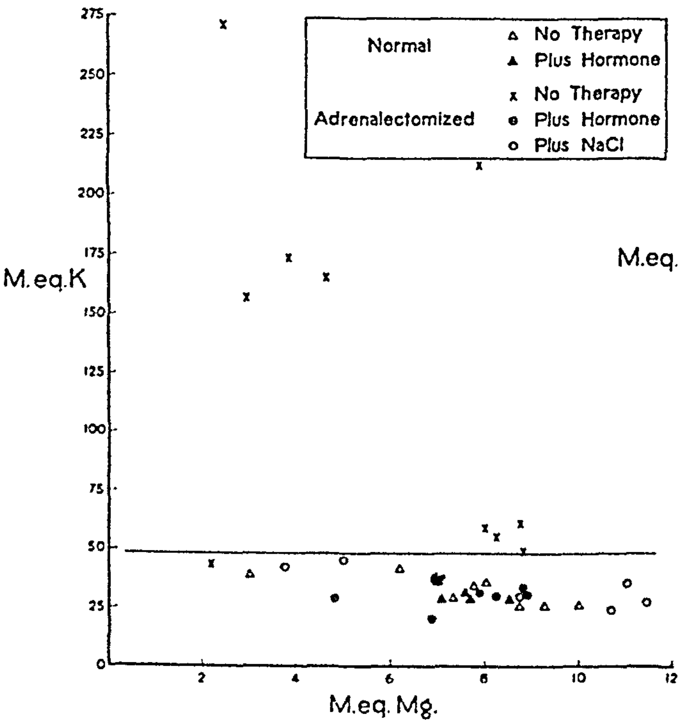

Fig. 1

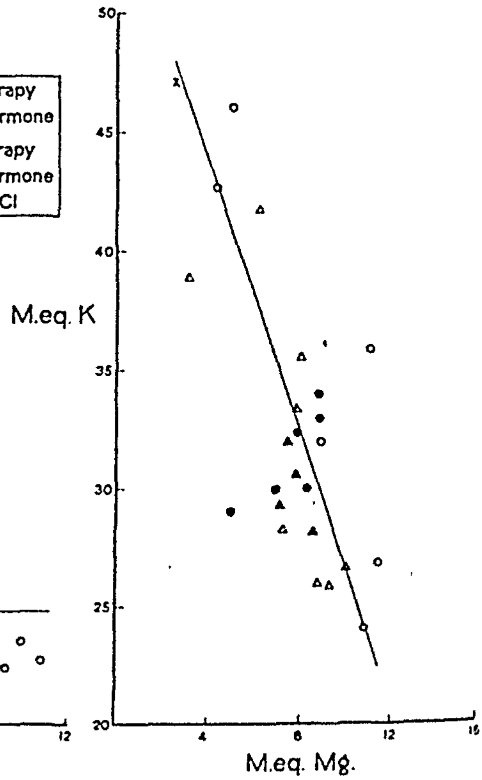

Fig. 2

Fig. 1. Gross relationship between potassium and magnesium in the whole skeletal muscles of all of the animals studied, values being reported as milliequivalents of cation per 100 grams of dry fat-free muscle. The hormone used was crystalline synthetic desoxycorticosterone acetate.

Fig. 2. Detailed relationship between potassium and magnesium in the whole skeletal muscles of all of the animals which were "compensated," values being reported as milliequivalents of cation per 100 grams of dry fat-free muscle. The hormone used was crystalline synthetic desoxycorticosterone acetate.

Variations in the muscle potassium of animals in adrenal insufficiency. Also, among the adrenalectomized animals which were given no specific therapy (fig. 1) the increases in muscle potassium were much greater in certain instances than were those found in our previous experiments with the four-hour fasted rats *in extremis*, or those reported by Harrison and Darrow. The animals with the highest muscle potassium values were not always those which were suffering from the most severe degree of insuffi-

ciency as judged by the weight loss. The suggestion is offered that the high muscle potassium might have been the result of the fact that these animals were still eating and therefore were still attempting to metabolize exogenous carbohydrate with the concomitant accumulation in the muscle cells of large quantities of impermeable anions which held potassium imprisoned with them, as a secondary effect. This concept is consistent with the experimental results of Buell, Strauss and Andrus (1) who demonstrated an accumulation of hexose monophosphate in the autolyzing muscle brei of cats in the final stages of adrenal insufficiency. It is a well known fact that animals in severe adrenal insufficiency do not eat voluntarily. It seems possible that inanition may be one means available to the animal of preventing further aggravation of a mounting chemical abnormality in its tissues. It is unfortunate that the quantities of blood available were insufficient for the determination of all of the cations in the serum of each animal. Although the potassium content of the pooled sera was increased above the normal value it gave no hint of the spectacular rise in muscle potassium in animals in adrenal insufficiency. Without tissue analyses this point would have been completely overlooked.

Variations in total base. Another feature of the results was the constancy of the *average value* calculated for the total base in the muscle in all of the groups studied except the group of animals in adrenal insufficiency (table 1). The constancy of this figure is more apparent than real, however, for there were significant variations among individual animals in the same group. For example, the maximum and minimum values in a group of eight normals were 59 and 45 meq., respectively. Variations among individual animals in the other groups were similar. It is recognized that the apparent variations may be greater than, or less than, the actual variations, due to the cumulative or compensatory experimental errors involved in the four separate determinations. Nevertheless, variations *within certain limits* might be expected on the basis of the changing magnitude of the "metabolic potassium" and the limitations of the mechanisms, which were available for the simultaneous adjustment of the other cations. It was only when the total base rose above the value of 62 meq. per 100 grams of dry fat-free muscle that the effects of adrenalectomy were uncompensated and there was serious adrenal insufficiency. It appears that it is a matter of vital importance to the animal to maintain in the skeletal muscle the total base concentration within rather narrow and well-defined limits. With appropriate dosage of sodium chloride or of desoxycorticosterone acetate the effects of adrenalectomy are compensated to a degree which is compatible with life, and it is only in an emergency such as a prolonged fast, vigorous exercise or an infection that such metabolic defects as low liver glycogen, hypoglycemia, and certain altered cation relationships become limiting factors in the animal's survival. In the

absence of specific therapy the potassium content of the muscle soon mounts, and when it reaches a value of 50 meq. per 100 grams of dry, fat-free muscle, effective compensation ceases abruptly.

Relationship of potassium to magnesium. It was reported by Harrison and Darrow (3) that the increase of potassium within the cell in the muscles of rats with adrenal insufficiency "is not associated with any change in the concentration of magnesium which is the other base found within the cells in considerable amounts". Figures 1 and 2 illustrate our experience in this connection. Fortunately, for the sake of simplicity of interpretation, both potassium and magnesium are predominantly cellular elements. It will be seen in figure 1 that the points tend to cluster along two distinct and unrelated curves, the lower curve being fairly well defined, the upper curve less well defined. The points falling on the lower curve all represent potassium values less than 50 meq. per 100 grams of dry, fat-free muscle and include all of the animals in all of the groups studied, except the group of animals in adrenal insufficiency[7]. That is to say, when the adrenal insufficiency was "compensated" by one means or another, the potassium content of the muscle remained below the critical level of 50 meq. and there was an orderly relationship between potassium and magnesium. When the insufficiency was uncompensated, however, the potassium content of the muscle rose. Suddenly, at a value of 50 meq. of potassium, as though there might have been a break in cell membranes due to pressure, there was a break in the linear relationship between potassium and magnesium.

The linear relationship between potassium and magnesium in the compensated animals is seen, in figure 2, to be such that for an increase of 10 meq. of potassium there was, *on an average*, a decrease of 3.5 meq. of magnesium, regardless of the method by which compensation was attained. It transpires that this mechanism of partial (about one-third) compensation of the effects of increases in the "metabolic potassium" by decrease in magnesium is quantitatively the principal adjustment made directly among the cations by means of which the total base is kept within the limits found normally.

Relationship of potassium to sodium. Figure 3 illustrates the fact that the points representing potassium-sodium relationships also fall into two categories, separated by a line representing a potassium value of 50 meq.: 1, the uncompensated condition in which the potassium was high and the relationship between the cations was irregular, and 2, the compensated condition in which the relationship between these cations may be seen in

[7] It will be seen that one point representing an animal in this group fell on the lower curve. In this one case the potassium content of the muscle happened to be below the critical level, and the adrenal insufficiency was compensated, at least for the moment.

more detail in figure 4. Normally muscle potassium is predominantly a cellular element, the quantity of which appears to vary considerably in contrast to muscle sodium which is predominantly an extracellular element, the quantity of which appears to remain fairly constant[8]. It is seen that in figure 4 all of the points fall on or near one of the two curves; *a*, the vertical line representing the normal untreated animals, in which the po-

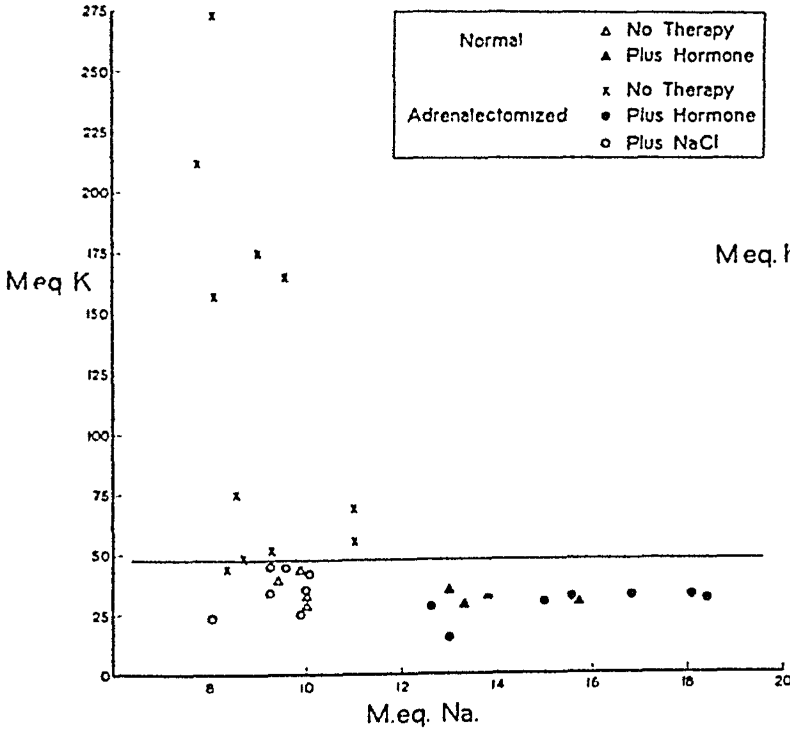

Fig. 3

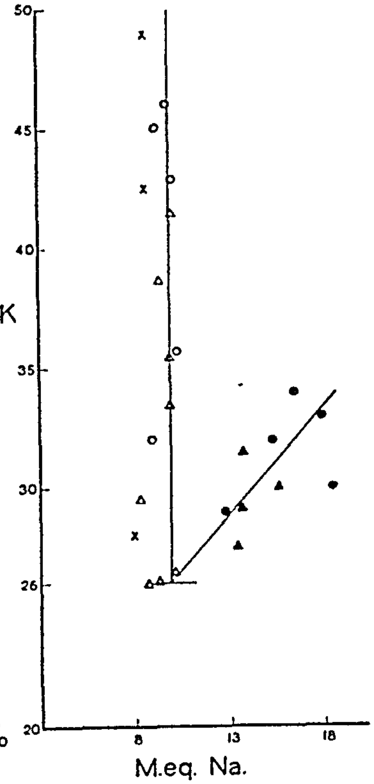

Fig. 4

Fig. 3. Gross relationship between potassium and sodium in the whole skeletal muscles of all of the animals studied, values being reported as milliequivalents of cation per 100 grams of dry fat-free muscle. The hormone used was crystalline synthetic desoxycorticosterone acetate.

Fig. 4. Detailed relationship between potassium and sodium in the whole skeletal muscles of all of the animals which were "compensated," values being reported as milliequivalents of cation per 100 grams of dry fat-free muscle. The hormone used was crystalline synthetic desoxycorticosterone acetate.

tassium appeared to vary independently of the sodium, and *b*, the sloping line in which the potassium and sodium appeared to vary together in nearly equivalent quantities, above a certain minimum level. In group *a* are found the normal untreated animals, the adrenalectomized animals

[8] Heppel (6) however, found that, if rats were deprived of potassium, the sodium content of the muscles was increased two-fold and that most of the sodium in such muscle was intracellular.

which were given sodium chloride, and also two temporarily compensated adrenalectomized animals to which no therapy was given. In these fifteen animals the sodium values varied only between 8.1 and 10.5 meq.

In group *b* are found the animals, both normal and adrenalectomized, which were treated with desoxycorticosterone acetate. The sodium and potassium values of these animals should be considered 1, in comparison with the corresponding values of the same types of animals which had not been treated with the hormone, and 2, in their relationship to each other. First, in the normal animals, treated with hormone, the values for muscle sodium were definitely increased beyond the limit of experimental error and probably beyond an increase in sodium which might have resulted from an increase in the volume of extracellular fluid. There can be little doubt that some sodium ions, in excess of the normal amount, have entered the cells. It will be seen that this effect is qualitatively the same but quantitatively greater in the muscles of the adrenalectomized animals, each given one pellet of the hormone. Larger doses or the same dose over a longer period of time might well have resulted in an even more exaggerated effect[9, 10].

The effect of desoxycorticosterone acetate on muscle potassium in normal animals was equivocal, probably only because it was small; in adrenalectomized animals the effect was dramatic. The average values for muscle potassium for the normal untreated animals was 32 meq. (maximum 42, minimum 26), for the normal treated animals was 30 meq. (maximum 32, minimum 28); for the adrenalectomized untreated animals 118 meq. (maximum 270, minimum 50); for adrenalectomlzed treated animals 30 meq. (maximum 34, minimum 20). There seems to be no room for doubt that the net effect of the treatment with desoxycorticosterone acetate in the adrenalectomized animals has been 1, the entrance of sodium ions into the muscle cells, and 2, the exit of potassium ions from the muscle cells.

As to the relationship between the sodium and potassium ions in the muscles of all of the animals which were treated with desoxycorticosterone acetate, figure 4 suggests several interesting points. The sloping line, representing this relationship, bisects the vertical line, representing the same relationship in normal muscles, at a point which coincides with the lowest observed value for potassium in normal muscle, which as explained

[9] Recently Miller and Darrow (7) reported decreases in muscle potassium and increases in muscle sodium when normal rats were injected subcutaneously with desoxycorticosterone acetate.

[10] The pellets remained *in situ* for too short a time to permit an accurate determination of the daily dose absorbed by each animal by reweighing the pellets after their removal. By comparison with results obtained in this laboratory (8) with dogs and patients, it may be assumed that approximately 0.3 mgm. of the hormone was absorbed daily by each rat. This dose is equivalent to about 0.5 mgm. injected in oil.

above, has been regarded as a basal value. Furthermore, at least in the few experiments at hand, the sum of the sodium and potassium values seen on the sloping line is in no instance greater than the largest value for "metabolic potassium" in the normal animals. Also, the slope of this line indicates that for values above the basal condition approximately equivalent amounts of sodium and potassium are involved.

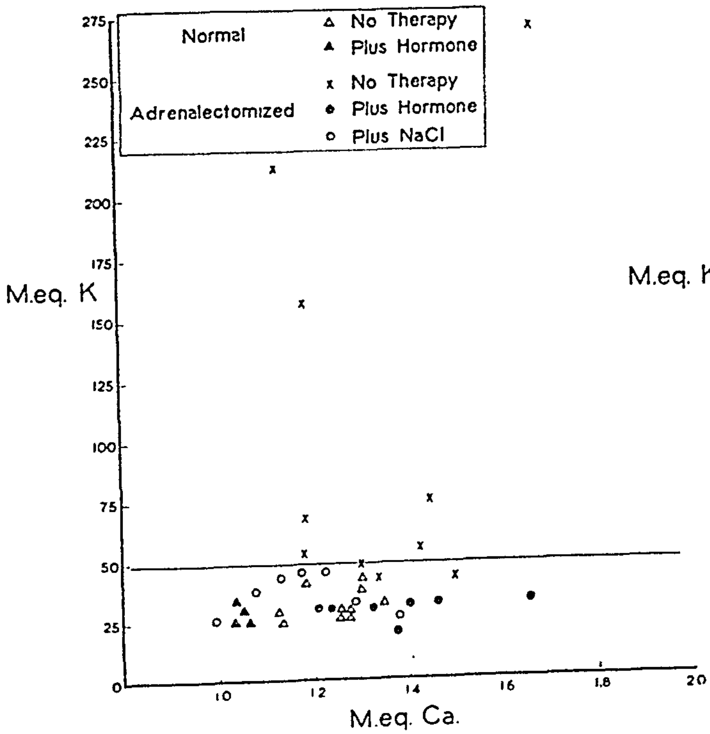

Fig. 5

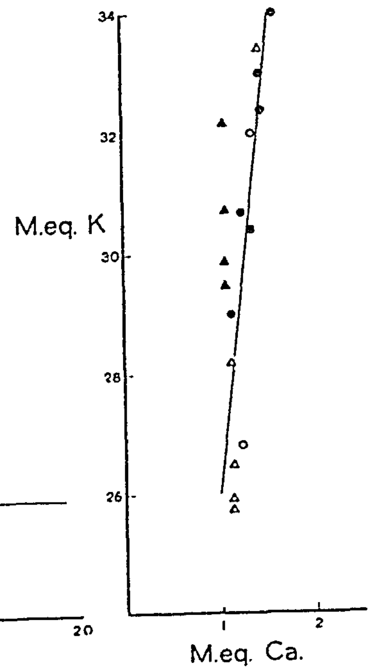

Fig. 6

Fig. 5. Gross relationship between potassium and calcium in the whole skeletal muscles of all of the animals studied, values being reported as milliequivalents of cation per 100 grams of dry fat-free muscle. The hormone used was crystalline synthetic desoxycorticosterone acetate.

Fig. 6. Detailed relationship between potassium and calcium in the whole skeletal muscles of the adrenalectomized animals which had been treated with desoxycorticosterone acetate.

Relationship of potassium to calcium. In our earlier work (1936) a higher calcium content was found in the pooled muscles from several four-hour fasted rats which were in the final stages of adrenal insufficiency, than in the pooled muscles from normal animals. More recently, when the muscles of individual unfasted animals in a milder degree of adrenal insufficiency were analyzed, as is shown in figure 5, high calcium values were observed occasionally but there was no demonstrable correlation with the high potassium values. Even when the insufficiency was controlled, i.e., when the potassium value was below 50 meq., the calcium appeared to

vary almost independently of the potassium. Failure to demonstrate a clean-cut correlation was to be expected, however, because the total quantity of calcium present in muscle was so small as to make its precise determination difficult and the maximum variation among all of the animals studied in this series was less than 1 meq. of calcium per 100 grams of dry, fat-free muscle. The picture is further complicated by the fact that as much as one-fourth of the total calcium in normal muscle is extracellular, a distribution which might mask trends which would otherwise be apparent. Only in the muscles of the adrenalectomized animals receiving desoxycorticosterone acetate but not in the muscles of normal animals, similarly treated, was there a suggested linear correlation between the potassium and calcium values (fig. 6). If future work proves this correlation to be real, it may possibly be more easily demonstrable in the adrenalectomized animals than in the normal, both receiving the hormone, because of the greater permeability of the cell membranes to calcium (and sodium) in the former and also to the smaller amounts of calcium in the serum and extracellular fluid of the former. In any event, for a rise of 10 meq. of potassium above the basal level, there was an apparent corresponding rise of about 0.6 meq. of calcium. The magnitude of the changes in calcium are such as to rule out the importance of these changes quantitatively in the maintenance of the normal value for total base.

General considerations. These experiments suggest new points of view on the puzzling and conflicting data in the literature concerning the part played by potassium not only in adrenal insufficiency but also in normal carbohydrate metabolism. A brief statement of the pertinent facts and theories which are based on good experimental evidence, is presented.

Whenever dehydration occurs, potassium tends to rise in the serum due to the inability of the kidney to excrete it. The total quantity of sodium available to the animal is an important factor in determining the degree of hydration and obviously will be influenced by *a*, the intake of sodium; *b*, the loss due to excretion by the kidney; *c*, the loss due to increased excretion by the intestine, (as in obstruction, diarrhea, etc.); *d*, the loss due directly to hemorrhage or indirectly to withdrawal of fluids into the tissues (as in shock, edema, etc.); *e*, the loss into the ascitic fluid after the experimental injection of glucose into the peritoneum. In all these cases where a decrease occurs in the total sodium content of the circulating fluids, an increase in the serum potassium may be expected and has been observed.

In normal carbohydrate metabolism serum potassium and sugar appear to rise and fall together, probably because potassium hexose phosphate is formed in the muscle cells. The amounts of hexose phosphate have been shown experimentally to be increased as the result of treatment with either insulin or epinephrin (9). On this line of reasoning, a low serum potassium would be expected (and has been observed) at appropriate time

intervals after the administration of either insulin or epinephrin. Lactic acid is formed as the end product of anaerobic metabolism and carbon dioxide and water as the end products of aerobic metabolism. These products escape from the cells, releasing potassium with them, and the potassium content of the serum is increased. This, *in our opinion*, may be one factor (together with potassium shifts resulting from anoxemia) which explains the high potassium content of the serum after exercise. Since the normal muscle cells contain all four cations, at least certain parts of the cell must have a degree of permeability to all four of them. There is little known definitely about the distribution of the cations in the cell compartments.

With this background, points suggested by these experiments may be discussed:

The muscle cells must be considered as an effective temporary reservoir for storing potassium and removing it from the serum, because of the large values found for the "metabolic potassium" and the large mass of muscle tissue in the animal. The total base of the muscle cells is kept within narrower limits than would otherwise be possible by a simultaneous though not equivalent shift of magnesium out of the muscle cells. It does not appear from the experimental evidence whether the extraordinary increase in muscle potassium in adrenal insufficiency is a primary effect, resulting from a lack of the adrenal cortex, or a secondary effect due to the retention of potassium ions in the cell to neutralize electrically some anions which may be accumulating in the cells in abnormal amounts. The latter possibility is consistent with the observations of Buell, Strauss and Andrus (1) who found *in the muscle brei* of adrenalectomized cats *in extremis* the formation of smaller than normal amounts of lactate (a permeable ion) and larger than normal amounts of hexose phosphate (an impermeable ion). These departures from the normal course of carbohydrate metabolism might be expected to be of greater magnitude in whole muscle or in muscle preparations in which the cell structure was maintained intact than in muscle brei. Interpreted in the light of the theory of Conway and Boyle (5), these facts suggest, but do not prove, that the rise in muscle potassium in adrenal insufficiency is an effect secondary to an error in the anaerobic phase of carbohydrate metabolism. If this is true the beneficial effect in the muscle of potent extracts of the whole adrenal cortex would be due to a correction of the abnormalities in the production of lactate. This hypothesis affords an explanation for the observations described by Kendall et al. (10) as follows: "The results of the injections of glucose . . . indicate that in the absence of cortin or adequate amounts of sodium and chloride the experimental animal becomes highly sensitive to potassium. It therefore seems probable that cortin modifies the rate of some chemical change which involves potassium".

Since the effect of desoxycorticosterone acetate on carbohydrate me-

tabolism is reported to be slight (11) its beneficial effect in compensating for the insufficiency following removal of the adrenal glands may be interpreted as being due to an alleviation of certain effects, rather than the cause, of the insufficiency. On the assumption that the effect of this hormone *in the muscle* is to increase the permeability of the cell membrane (the size of the pores, according to Conway and Boyle) the hexose phosphate anions which formerly accumulated in the cells and retained potassium ions with them might now escape and liberate the potassium cations with which they were associated. As a corollary the sodium ions (which are larger than potassium ions) would now be expected both to enter and to leave the cell indiscriminately with the potassium ions as demanded by the needs for "metabolic potassium", the laws of electrical neutrality, etc. (fig. 4). In this way not only would the tendency toward hypertonicity of the muscle cells in adrenal insufficiency be relieved thus aiding normal hydration of the tissues, but the muscle cells would be freed from the pharmacological effects of high concentrations of potassium ions. (They would, however, be submitted to larger quantities of sodium ions, and the potassium to sodium ratio would be lowered.) The potassium which was released from the muscle cells would temporarily increase the potassium content of the serum, but would readily be excreted in the urine, giving the well known potassium diuresis which occurs early in association with the treatment with desoxycorticosterone acetate (12). There have been reports in the literature (13) of periodic weakness produced in dogs by long continued overdosage with desoxycorticosterone, which was characterized by low serum potassium values and was alleviated by potassium salts and also (14) of transient paralysis and muscular weakness in a patient suffering from Addison's disease, treated daily with 25 mgm. of the hormone, following intravenous injections of sodium chloride and dextrose solutions before and after a nephrectomy. The ratio of potassium to sodium in the muscles, therefore, appears to be a matter not only of physiological interest but also of clinical importance.

The mode of action of sodium chloride in compensating for the specific effects of adrenal insufficiency in the muscle is not clear. Its mode of action on the kidney seems to be simple enough; by making good the loss of sodium and chloride it prevents the depletion of these elements in the plasma, and thus favors normal hydration, normal kidney function and normal serum potassium levels. How it attains the result of keeping the muscle cell potassium within normal limits is not revealed by these experiments. There is no evidence here of increased permeability of the cell membranes; in fact, there appears to be less sodium rather than more, inside the muscle cells. The fact remains however, that treatment with sodium chloride has in some way prevented the increase in muscle potassium which would have occurred in its absence.

The immediate causes of the low blood sugar in adrenal insufficiency are to be sought *a*, in the liver, where there is an impaired ability to synthesize glycogen either from d. lactic acid (2) or from certain metabolically equivalent compounds, such as pyruvic (11) acid which may be secondarily formed from other metabolites arising from protein catabolism, and *b*, in the muscles where there is an impaired ability to form lactic acid (1). A vicious cycle is instituted with respect to the carbohydrate cycle. The injection of intravenous glucose into an animal in acute adrenal insufficiency might benefit the condition not only by relieving the hypoglycemia directly but also by providing a source for the synthesis of liver glycogen (15). Unless some provision were also made for relieving the accumulation of potassium salts already present in the muscle (such as intravenous salt, cortical extract, etc.) this added glucose would take potassium with it into the muscle cells and further aggravate a situation which was already bad. These facts explain the common observation of the effect of intravenous glucose in the treatment of patients suffering from Addison's disease, of a temporary relief of the symptoms, followed shortly by the precipitation of a crisis.

Potassium accumulates in the serum because of *a*, failure on the part of the kidney to excrete it readily because of the dehydration; *b*, failure on the part of the liver to deposit it, as is usually done at least in small quantities, when glycogen is synthesized (16), and *c*, failure on the part of the muscles to take up additional quantities of potassium ions readily against the high gradient of potassium ions already present in the muscle cells. All of these factors act together to institute a vicious cycle with respect to potassium. The result is that in adrenal insufficiency the blood sugar remains low and the plasma potassium remains high in contrast to the normal condition, where they vary together.

These considerations explain why potassium is abnormally toxic in adrenal insufficiency.

It becomes apparent that it is difficult, if not impossible, to dissociate completely the errors in carbohydrate metabolism in adrenal insufficiency from those in electrolyte balance since normal carbohydrate metabolism is intimately associated with changes in the distribution of certain electrolytes. When adrenalectomized animals are "kept in good condition" by one means or another, in order better to study the errors inherent in adrenal insufficiency *per se* unknown factors may unwittingly be introduced which may alter the problem and may thus confuse the issue instead of clarifying it.

SUMMARY AND CONCLUSIONS

1. The skeletal muscles of individual unfasted male rats have been analyzed for moisture, lipid, sodium, potassium, calcium and magnesium.

The animals were studied in the following conditions: 1, normal with no therapy; 2, normal treated with desoxycorticosterone acetate; 3, adrenalectomized treated with sodium chloride; 4, adrenalectomized treated with desoxycorticosterone acetate, and 5, untreated adrenalectomized in varying degrees of insufficiency. Pooled sera from individual animals in each of these groups were analyzed for sodium, potassium, calcium, chloride and non-protein-nitrogen.

2. The potassium content of the muscles of normal unfasted animals varied widely, probably in relation to the demands of carbohydrate metabolism. The potassium content of the muscles of animals in adrenal insufficiency was very high and very variable. This point has been discussed.

3. The average total base of the muscles of all of the groups was remarkably constant regardless of the method by means of which compensation was attained, with the exception of the group in adrenal insufficiency. There was considerable variation in total base among individual animals in the same group.

4. The relationships between muscle potassium and the other cations have been described. Below the critical potassium level there was an orderly relationship between potassium and the other ions. Above this level, i.e., in adrenal insufficiency, this relationship was abruptly changed.

5. Below the critical potassium level, for every 10 meq. of potassium gained by the muscle, on the average, 3.5 meq. of magnesium were lost.

6. Desoxycorticosterone acetate, when administered under the conditions described, caused the entrance of small quantities of sodium into the muscle cells of normal animals and of larger quantities of sodium (and perhaps of a little calcium) into the muscle cells of the adrenalectomized animals. This hormone prevented the rise in muscle potassium which would have occurred in the adrenalectomized animals in its absence. A possible mechanism for this effect is discussed.

7. Sodium chloride likewise prevented the rise in muscle potassium which would have occurred in its absence in the adrenalectomized animals. There was no evidence of increased permeability of the muscle cells in this group of animals.

8. The probable importance of the ratio of potassium to sodium in the muscle cell has been pointed out.

9. The correlation of these new observations in the interpretation of old problems associated with both adrenal insufficiency and carbohydrate metabolism has been discussed.

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THE RENAL EXCRETION OF AN ANTIDIURETIC SUBSTANCE BY THE DOG

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The normal water exchange of most laboratory mammals depends upon the presence of the pars nervosa of the pituitary. Transection of the pituitary stalk or destruction of the supra-optic nuclei in the hypothalamus results in atrophy of the pars nervosa and an increase in water exchange; administration of extracts of the pars nervosa will then reduce the water exchange to its normal level. While these facts have often been demonstrated, the rôle of the pars nervosa in the regulation of water exchange in the intact animal is only vaguely defined.

Gilman and Goodman (1) discovered an antidiuretic material in the urine of normal dehydrated rats. They concluded that this substance was of pituitary origin since it was not found in the urine of dehydrated rats after hypophysectomy. Walker (2), in a more comprehensive investigation, tried to find "an antidiuretic hormone in body fluids in concentrations which vary with physiological variations in urine volume". Although some of his experiments confirmed Gilman and Goodman, discrepancies in others caused him to doubt their conclusions. Assays by Ingram, Ladd, and Benbow (3) of urine from dehydrated normal and diabetes insipidus cats provide strong evidence that the excretion of an antidiuretic material by the cat depends upon the presence of the pars nervosa.

We have assayed the urine, blood and pars nervosa of normal and diabetes insipidus dogs after dehydration. The method of assay (4) differs from that used by other investigators. As a test animal we have used the diabetes insipidus dog which will maintain a diuresis for several hours. The criterion of antidiuresis is not simply a decrease in urine flow, but an increase in urine concentration. Since urine flow is the resultant of two independent functions, glomerular filtration and tubular reabsorption of water, it is essential in evaluating antidiuretic effects to consider each separately. This is especially important since Burgess, Harvey and Marshall (5) have shown that an extract of the pars nervosa effects an antidiuresis by increasing tubular reabsorption of water.

The volume of glomerular filtrate was measured by the renal clearance of creatinine, since there is good evidence that exogenous creatinine is excreted

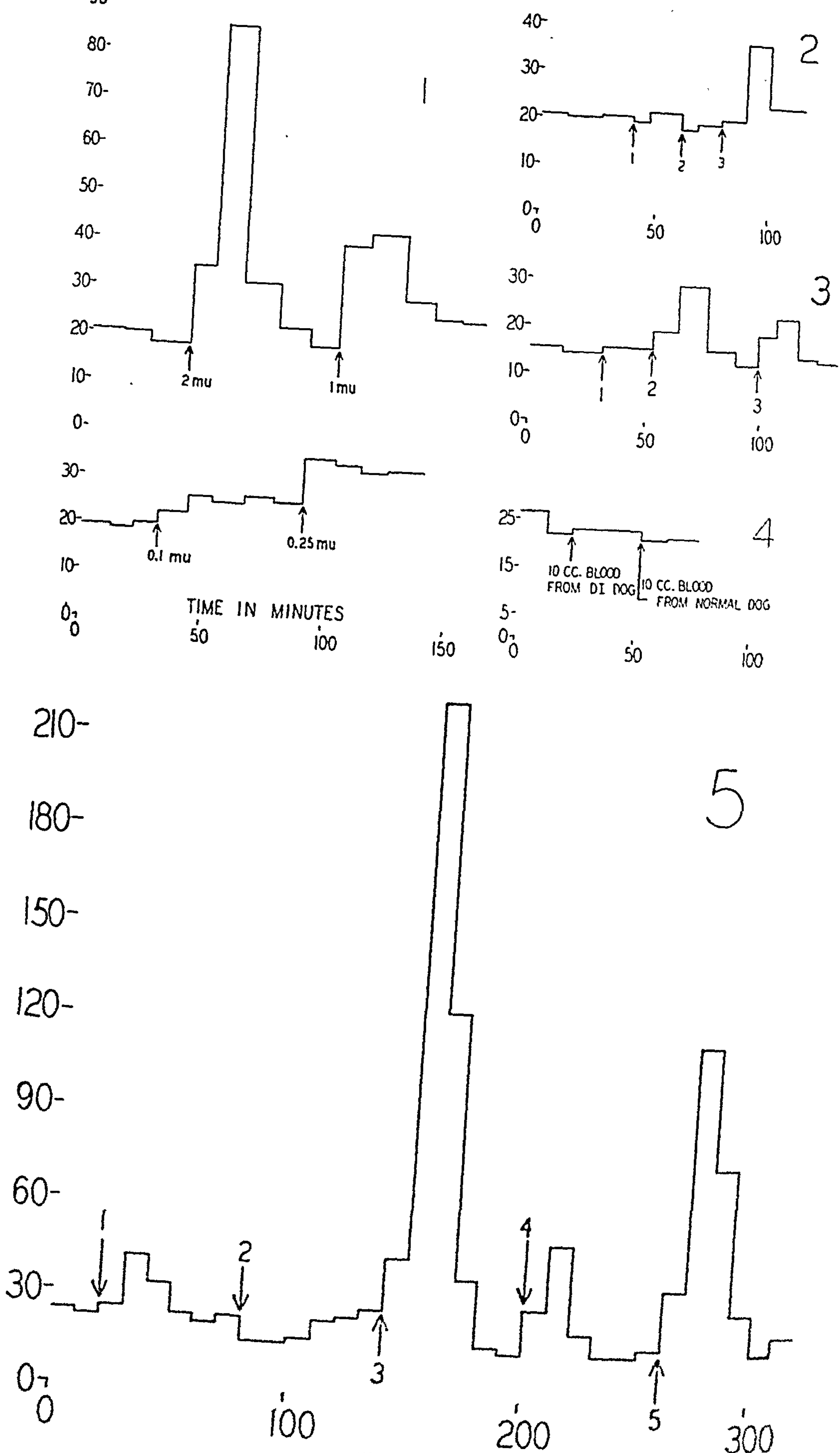
solely by glomerular filtration in the dog (6, 7). The creatinine U/P ratio, or the number of cubic centimeters of plasma required to produce 1 cc. of urine, was used as a measure of water reabsorption. One-tenth of a milliunit of Pitressin (Parke-Davis) produced a measurable increase in creatinine U/P ratio (fig. 1).

Intravenous injection of the material to be assayed was used for two reasons. First, the rates of absorption have been shown to vary greatly after subcutaneous injection (8), and in our assays duration of the antidiuresis was an important part of the response. Second, since large volumes of fluid may be given intravenously, it was unnecessary to concentrate the urine by procedures which might alter its constituents (1, 2, 3, 9). In some assays as much as 100 cc. of fresh untreated urine were injected.

METHOD. Five grams of creatinine in 100 cc. of water were given by stomach tube to the test animal (a diabetes insipidus dog) about 2 hours before the assay and about 18 hours after feeding. The dogs, all females, were accustomed to catheterization and venipunture and lay quietly on the board. Blood samples were collected at one hour intervals, centrifuged immediately, and duplicate aliquots of plasma precipitated with tungstic acid. The bladder was emptied by washing out with air and urines collected at carefully timed intervals, usually of 10 minutes' duration. Errors in volume measurement were minimized by collecting the urines in volumetric flasks which were then filled to the mark with distilled water from a burette. At least two control clearances were run in every experiment before any injections were given. Creatinine concentrations were determined by the alkaline picrate method, and the clearance and U/P ratio, calculated. The accuracy of the method was improved by using a photoelectric colorimeter and by developing the color of the solutions in a room maintained at a constant temperature.

Donor animals were normal dogs and dogs which had a permanent polyuria as the result of pituitary stalk section. Blood and urine samples for assay were collected from these dogs after a water deprivation of 2 to 4 days. The dogs were catheterized and the bladder washed out with 0.9 per cent saline. After an interval of 15 minutes to 2½ hours, the urine was collected, the bladder again washed with saline and the washing added to the urine. The specimens thus collected were diluted to the same volume and injected intravenously into the test animal. In a few cases, blood samples for assay were also collected. Ten or twenty cubic centimeters of blood were withdrawn from the external jugular vein through a 15 gauge needle and immediately injected into the test animal. No anticoagulant was used.

In one experiment the donor dogs were killed immediately after the urine collection, and the pars nervosa of the pituitaries removed. Each



1
2 mu
1 mu
2
3
0.1 mu
0.25 mu
TIME IN MINUTES
4
10 CC. BLOOD FROM DI DOG
10 CC. BLOOD FROM NORMAL DOG
5

Figs. 1-5

gland was thoroughly macerated with sea sand in saline and an aliquot of this extract assayed in the same manner as the urines. Assays were also done on urine samples collected from diabetes insipidus dogs before and after injection of fresh saline extracts of posterior lobes.

RESULTS. The urine from dehydrated normal dogs invariably caused an antidiuresis when injected into a diabetes insipidus dog. This antidiuresis was the result of an increase in the concentration of the urine as expressed by the creatinine U/P ratio (figs. 2 and 3). The urine from dehydrated diabetes insipidus dogs never produced an antidiuresis; in 6 out of 7 cases, it was followed by a slight diuresis. The blood from normal and diabetes insipidus dogs never produced any effect on creatinine clearance or U/P ratio in the test animal (fig. 4). This finding is apparently contradictory to Melville's results (10). The discrepancy may be accounted for in three ways: Melville made an extract of the blood; he used a larger volume (50 cc.); and since he did not assay the blood of the hypophysectomized dog, the substance he found may be not be of pituitary origin. Since 0.1 milliunit of Pitressin was easily detected by our method, the blood assayed in this experiment must have contained less than the equivalent of that amount in 10 cc. When the posterior lobes of the donor dogs were assayed, the response of 1/10 of the diabetes insipidus gland was less than half of the smaller response to 1/500 of the normal posterior lobe (fig. 5). The antidiuretic potency of the diabetes insipidus posterior lobe was therefore less than 2 per cent of the normal.

Fig. 1. Two experiments were done on a diabetes insipidus dog to determine the threshold dose of Pitressin. The upper line indicates the responses to 2 and 1 milliunit doses; the lower line, the responses to $\frac{1}{16}$ and $\frac{1}{4}$ milliunit doses. In all figures the ordinate is time in minutes; the abscissa, creatinine U/P ratio.

Fig. 2. Assays of 30 minute urine specimens from 2 diabetes insipidus and 1 normal dog deprived of drinking water for 97 hours. All specimens were diluted to 20 cc. with isotonic saline and injected intravenously. The urines from the polyuric dogs were injected at the first and second arrows; the urine from the normal dog at the third arrow. Only the last caused an antidiuresis.

Fig. 3. Assay of 30 minute urine specimens from 1 diabetes insipidus and 2 normal dogs dehydrated for 47 hours. Each urine was diluted to 20 cc. with isotonic saline and injected intravenously. The diabetes insipidus urine was injected at the first arrow; the urine from the normal dogs at the second and third arrows.

Fig. 4. Assay of blood from normal and diabetes insipidus dogs. Neither sample of blood contained a detectable amount of antidiuretic substance.

Fig. 5. Assay of urines and pituitaries of a normal and of a diabetes insipidus dog. After the dogs had gone without water for 68 hours, $2\frac{1}{2}$ hour urine specimens were collected from each. The urine from the normal dog was injected at the first arrow; the urine from the diabetes insipidus dog at the second arrow. After the urine collection, the dogs were killed, their pituitaries removed and macerated in saline. At the third arrow $\frac{1}{500}$ of the normal dog posterior lobe was injected; at the fourth arrow $\frac{1}{10}$ of the posterior lobe of the diabetes insipidus dog was injected. The fifth injection is a repetition of the third.

Although urine samples from the untreated diabetes insipidus dog had no antidiuretic effect, urine samples taken from the same dog after the injection of a saline extract of pars nervosa had a marked antidiuretic effect. This result shows that the kidney of the diabetes insipidus dog, like that of the normal rabbit (11) is capable of excreting an antidiuretic substance.

CONCLUSIONS

1. The urine of the dehydrated normal dog contains an antidiuretic substance; the urine of the dehydrated diabetes insipidus dog contains none.
2. If there is any antidiuretic substance in the circulating blood, it is present in concentrations equivalent to less than one milliunit of Pitressin per 100 cc.
3. The kidney of the diabetes insipidus dog is capable of excreting an antidiuretic substance when the material is administered by intravenous injection.
4. The absence of an antidiuretic substance from the urine of the dehydrated diabetes insipidus dog is associated with an enormously diminished antidiuretic content of the pars nervosa.

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MODIFICATION OF THE PANCREATIC RESPONSE TO SECRETIN BY URINE AND URINE CONCENTRATES

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We have noted (1) that blood serum has the property of inactivating secretin and that such inactivation is on an enzymic basis; furthermore, that although secretin is inactivated by gastric and pancreatic juice and by crude extracts of pepsin and trypsin, it is unaffected by these enzymes in crystalline form. On the strength of these findings it was postulated that the ineffectiveness of orally administered secretin was due to the presence of secretinase in the gastrointestinal secretions which destroy the hormone before it can be absorbed. Hence it appeared not improbable that secretinase was rather widely distributed in the body and that the question of its occurrence in the urine merited investigation. Moreover, a demonstration of secretinase in the urine would afford a logical explanation for the circumstance that secretin has never been found in urine or urine concentrates.

Consequently a series of experiments was conducted with a view to answering this question. In the course of this work, it became apparent that when the animals were given a large amount of urine or urine concentrate the pancreas became increasingly refractory to control injections of secretin; the agent causing this refractoriness appeared to be present in both unboiled and boiled material. This indicated the presence in the urine of a thermostable substance which inhibits the secretory activity of the pancreas. That such a circumstance is not unlikely is indicated by the studies of several investigators (2–6) who have demonstrated that the urine contains a gastric secretory depressant.

In other words, we considered the possibilities that the urine contains substances which may act on the hormone itself and on the end-organ excited by the hormone. These possibilities were examined in the experiments detailed below.

Experimental. 1. *Materials.* Human urine served as the source material. It was used in the following forms: untreated; vacuum distilled and dialyzed; the precipitate obtained by the addition of 10 volumes of acetone to vacuum distilled dialyzed urine; and the benzoic acid adsorbate

according to the method of Katzmann and Doisy (7) suspended in water, boiled, filtered, and precipitated with acetone. Various other reagents were tried and found of no use. The dry preparations were injected in 100-mgm. doses.

2. *Secretinase in the urine.* The animal preparations and secretin concentrates were the same as those we employed in our previous studies (1). The presence of secretinase was tested by incubating freshly voided human urine with the standard amount (1 mgm.) of secretin concentrate and comparing the response to that elicited by a standard dose of secretin and by a mixture of secretin with boiled and cooled urine similarly incubated. The urine concentrates were assayed for secretinase in a similar manner.

3. *Pancreas inhibitor in the urine.* The same animal preparations were used except that in some dogs the secretin was injected in dilute solution into the femoral vein by a Woodyatt pump at a very slow and constant rate (0.1 mgm. per min.) in order to stimulate a slow and regular flow of pancreatic juice from the cannulated duct. When such a flow was established one of the urine concentrates was injected and pancreatic flow recorded for one to two hours thereafter. In most of the dogs the pancreatic response to the standard dose of secretin was ascertained, following which the urine concentrate was injected. At subsequent intervals the secretin administration was repeated and the pancreatic response noted with reference to the control.

RESULTS. 1. *Urinary secretinase.* In all cases in which secretin was incubated with freshly voided normal urine a definite inactivation of the secretin occurred, whereas there was no inactivation when previously boiled and cooled urine was used. The results are listed in table 1.

2. *Pancreas inhibitor.* Indication of the presence of this agent was obtained when *urine concentrates* were incubated with secretin. It was observed that there was an apparent inactivation by both boiled and unboiled urine concentrates, and furthermore, the response to control injections was definitely less for some time after they were administered. The data illustrating these findings are listed in table 2.

When the urine concentrates were injected intravenously into dogs secreting at a uniform rate in response to continuous secretin injection, a retardation of the secretion was noted. Such retardation was marked when the secretory rate of the pancreas was slow, and was minimal when the gland secreted at a rapid rate. Illustrative records are shown in figure 1. Throughout these experiments complications were encountered in about half the dogs in the form of a drop in blood pressure which was mild in degree (30 mm. Hg or less), gradual in onset, and up to 20 minutes in duration. It was to avoid this that the procedure was altered to that of standardizing the animal to a single dose of secretin, then injecting the urine concentrate, waiting for recovery from any consequent hypotensive

TABLE 1

Enzymic inactivation of secretin; alterations in response to a standard dose of secretin following incubation with 5 cc. of urine

DOG	PROCEDURE	INCUBATION TIME	RESPONSE (DROPS)	CONTROL (DROPS)
		hours		
1	Secretin + urine	4	11	27
2	Secretin + urine	3	14	31
	Secretin + boiled urine	3	26	
3	Secretin + urine	3	7	20
	Secretin + boiled urine	3	21	
	Secretin + urine	$5\frac{1}{2}$	6	29
	Secretin + boiled urine	$5\frac{1}{2}$	27	
4	Secretin + dialyzed urine	4	0	35
	Secretin + boiled dial	4	24	
5	Secretin + urine	4	16	33
	Secretin + boiled urine	$2\frac{1}{2}$	33	
	Secretin + urine	$4\frac{1}{2}$	14	36
	Secretin + boiled urine	$4\frac{1}{2}$	31	
6	Secretin + urine	24	7	75
	Secretin + boiled urine	24	45	
7	Secretin + urine	3	6	28
	Secretin + boiled urine	3	26	
8	Secretin + urine	4	0	12
	Secretin + boiled urine	4	10	
9	Secretin + urine	5	36	84
	Secretin + boiled urine	5	78	
10	Secretin + urine	4	12	32
	Secretin + boiled urine	4	27	

TABLE 2

Refractoriness to secretin after injection of urine concentrates

INJECTION	RESPONSE (DROPS)	
	Dog 11	Dog 12
Control (1 mgm.)	42	37
Secretin + dialyzed urine	0	3
Secretin + boiled dialyzed urine	9	11
Secretin + acetone ppt. from dialyzed urine	0	0
Secretin + boiled acetone ppt. from dialyzed urine	0	6
Control	17	8
Control after 1 hour		37
Control 1 minute after acetone ppt		1

effect, and finally repeating the standard secretin injection at intervals. In all animals administration of the urine concentrate was followed by a relatively refractory state of the pancreas, which was maximal in 15 to 20 minutes, gradually diminished, and had usually disappeared in two hours. The data are listed in table 3.

DISCUSSION. The results of the assays for secretinase in the urine are self-explanatory. It is apparent from the data obtained that there exists

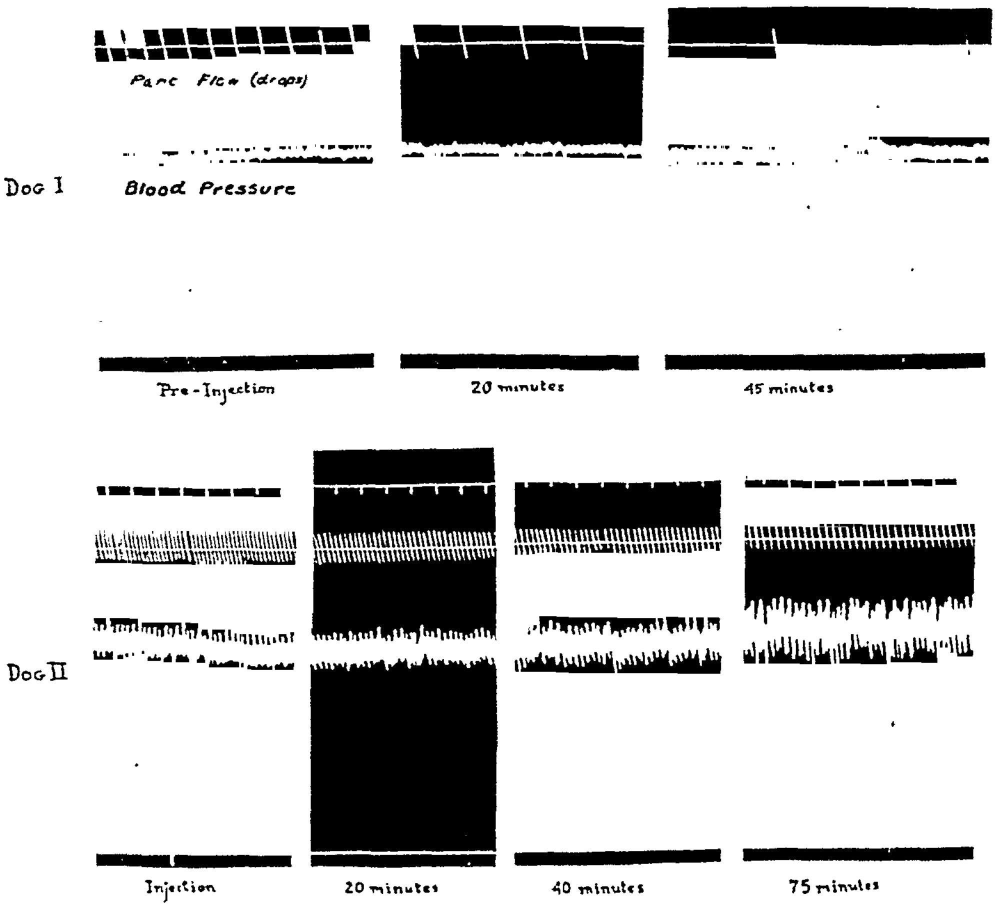

Fig. 1. Pancreatic secretion in response to continuous injection of secretin, 0.1 mgm. per minute, showing retardation of secretion following intravenous injection of urine concentrate.

in the urine a thermolabile substance which inactivates secretin. Whether this enzyme is identical with the secretinase demonstrated to exist in blood serum is problematical. The other principle demonstrated to be present in the urine is heat-stable, non-diffusible, and exerts its effect directly on the pancreas. Whether this effect is a direct inhibition of the secretory activity of the pancreatic acinar cells, or whether it is primarily vascular in nature, cannot be answered until a satisfactory method is developed for measuring blood flow through the pancreas.

Two circumstances argue against the likelihood of a vascular mechanism. In the first place, the hypotensive effect of the urine concentrates was inconstant and in half the animals was entirely absent. Secondly, in three of the animals which had evidenced a fall in blood pressure, the hypotensive effect was controlled by injections of peptone. To do this, a sufficient interval was allowed to elapse after administration of the urine concentrate until the pancreas had regained its normal activity, after which 2 cc. of 10 per cent Witte's peptone was injected. Following recovery, which took place in 5 to 10 minutes, the secretin injection was repeated and the response found to be identical with that obtained prior to the peptone injection. It was concluded from these observations that a transitory fall

TABLE 3

Pancreatic response in drops to a standard dose of secretin before and after injection of a urine concentrate

DOG NO.	BEFORE		AFTER											
	Control response		Time in minutes											
			10	20	30	40	50	60	70	80	90	100	110	120
13	23	23	14 15		5			8				10		17*
14	21	20	17	12		14				18	17			
	17		13	9			13			13				
15	27	25	13	13		15			21		24			
16	36		2		8	13		24						
17	30			15		19	21	28						
18	31	32		24		20	18	28			31			
		31	18		15			16					25	
19	13	13		1							16		25	25
20	32				13		19		25		32		32	
21	46	48	32		18		16		24	35	40			42
21	50		32		26		15	18			35			44

* Four hours.

in blood pressure *per se* has no lasting effect on pancreatic secretory activity.

The effect on pancreatic secretion cannot be a result of contamination with pyrogen, since the urine concentrate was prepared under precautions designed to eliminate bacterial growth and was free of demonstrable pyrogenic effect. A by-product very high in pyrogen, supplied us by Doctor Gray, failed to show any inhibitory effect on the pancreas.

The agent in the urine responsible for the vaso-depressor effect of the concentrates is obscure. The only material which has been characterized as having such an action is callicrein (8), which is heat-labile and therefore could not be the factor in the urine concentrates used in this work, which had been boiled in the process of preparation. Moreover, the hypotensive

action of callicrein is rapid in onset and brief in duration. Wollheim and Lange (9) and Wollheim (10) have noted a vasodepressor activity in extracts from urine and from posterior lobe of the hypophysis which is very similar to that noted in this work, and which they ascribe to a thermostable substance termed "depressan" or "detonin".

The thermostable pancreatic inhibitor demonstrated to exist in the urine has been called *uropancreatone*. This is in conformity with the name urogastrone applied to the gastric inhibitory agent described by Gray *et al.* (loc. cit.). The present findings suggest the interesting possibility that the urine contains one or more principles which may regulate the production of several of the digestive secretions. The presence of such bodies in the urine obviously denotes their excretion; their original source remains to be established.

SUMMARY AND CONCLUSIONS

Assays of human urine for secretinase activity have been conducted and the presence of such an enzyme in the urine demonstrated. In addition, the urine has been shown to contain a thermostable principle which directly affects the secretory activity of the pancreas, and this is termed uropancreatone.

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ACTIVITY IN ISOLATED SYMPATHETIC GANGLIA

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REFLEXES IN SYMPATHETIC GANGLIA. A great variety of sympathetic responses may be obtained when stimuli are applied to afferent fibers from which the impulses are ultimately transmitted to efferent sympathetic neurons. If sensory neurons were present in the sympathetic ganglia, a reflex arc complete in the isolated ganglion would be a possibility. Dogiel (1) described such neurons, but his interpretation has been doubted by Langley (2), Ranson (3) and many others.

The physiological evidence for such a reflex mechanism is just as equivocal as the anatomical evidence, and many of the phenomena first thought to illustrate reflexes in the peripheral sympathetic ganglia have since been interpreted differently. According to Langley (2), "The peripheral neurons connected with a given spinal neuron are not necessarily all in one ganglion. . . They are connected with three, four, or more ganglia. This fact affords explanation of nearly all the 'reflex' actions which have been described as occurring in peripheral ganglia. The nervous impulse set up in one branch of the preganglionic fiber passes to the other branches, so to the peripheral ganglia, and to tissues more or less remote from the point stimulated. These reflexes may be called preganglionic axon reflexes" (pp. 11–12).

Study of the activity of the peripheral sympathetic system has been aided by the use of the galvanic skin reflex. It has been shown that the changes in the conductivity of the skin are most marked over the palmar and plantar surfaces of the hands and feet, and that these changes are effected largely, if not solely, through the sweat glands.

Using this technique, Schwartz (4) studied the sympathetic response in the pads of cats after the dorsal roots of the brachial plexus had been cut, and after the stellate ganglion had been cut off from the central nervous system. He concluded that "changes in the skin resistance in the pad of a cat's forepaw occur in response to reflex activity of the sympathetic nervous system. In the present study it is shown that a certain fraction of these reflex impulses are mediated solely through the stellate ganglion; that is, they are true sympathetic reflexes and require no central connection of the stellate ganglion. The afferent fiber carries impulses

from the blood vessels or deeply lying tissue of the forelimb, through the grey ramus, to its cell of origin in the stellate ganglion. There synaptic relations with the efferent neurons occur".

Magoun, Hare and Ranson (5), studying the effects of cerebellar stimulation upon the contractions of deafferented muscle, prepared some cats with the sensory innervation of the forelimb abolished. The dorsal roots of the fourth cervical through the second thoracic segments were cut according to the technique of Schwartz. Six cats were prepared in this manner, but when they were subjected to a careful sensory examination, it was found that each cat was still sensitive on the deafferented side over the posterior axillary fold, the dorsal surface of the arm, the posterior part of the elbow, and for about a centimeter distal to the elbow along the ulnar side of the forearm. Consequently, a second series of cats was prepared with the dorsal roots cut from the fourth cervical through the fifth thoracic segments. Sensory examination revealed total anesthesia of the affected forelimb. This observation aroused the suspicion that the activity which Schwartz had observed might be due to the stimulation of afferents of cerebro-spinal origin, which activated the sympathetic ganglia through the preganglionic fibers of the ventral roots.

The purpose of the following experiment was to test this possibility. The criterion of sympathetic activity used was a decrease in the resistance of the forepad to the flow of a galvanic current. The procedure of examination was as follows: the cat was fed, and immediately placed in a canvas strait-jacket, which was designed to avoid any interference with the circulation of the forelimbs. The cat was continuously petted by an attendant, and all of the observations reported were made when the cat was quiet. Leads were taken from the center pads with saline-zinc sulphate-zinc electrodes, held in place by strips of dental dam. If recordings were to be made from more than one pad, all the electrodes were fastened on at the beginning of the experiment, and the leads shifted from one to another. The low resistance electrode was usually applied to a region of the tail anesthetized with novocain and incised to eliminate skin resistance. In the examination of cats with deafferented forelimbs, the low resistance electrode was often placed on the anesthetized shoulder; it is essential that this electrode be placed on an anesthetized area, since the pain of application of saturated zinc sulphate solution to a fresh incision would cause such a burst of sympathetic activity, that an additional reflex discharge would be obscured. The resistance of the forepad was measured with a potentiometer (fig. 1) in which a variable resistor was balanced against the resistance of the cat. A change in skin resistance caused a deflection of the galvanometer which was sensitive enough to detect a change of less than one-tenth of one per cent. The resistance was measured between the electrode over the incised skin of the tail or shoulder and the

electrode on one of the forepads; that the greater part of this resistance was offered by the skin of the forepad was shown when a superficial scratch through the skin at this point caused the resistance of the cat to fall from 10–15,000 ohms to 1–2,000 ohms. Visual, auditory, and cutaneous stimuli were used. In a few cases the galvanic skin reflexes were recorded with a Hindle model string galvanometer.

Ten cats with dorsal roots C_4 through T_5 cut on the left side were repeatedly examined for sympathetic responses. Stimulation of the anesthetized limb, even when extremely severe (pinching with hemostats,

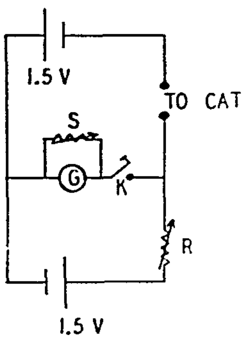

Fig. 1

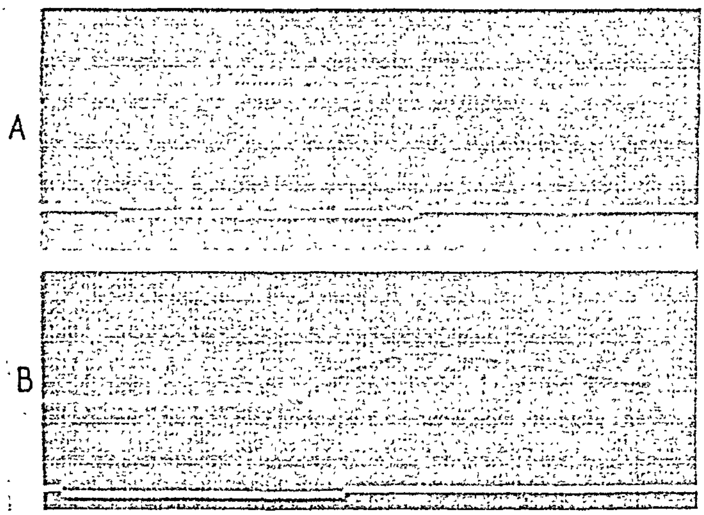

Fig. 2

Fig. 1. Potentiometer for measuring skin resistance. *G*, Leeds and Northrup galvanometer 2420-c.; *S*, shunt; *R*, one megohm decade resistor in steps of 100 ohms; *K*, switch.

Fig. 2. String galvanometer recordings of skin resistance. (Recorded by Dr. Dayton J. Edwards.)

A. Absence of change in left fore pad on crushing the left elbow with heavy forceps.

B. Fall in skin resistance in right pad on pinching the cat's tail.

Lower line indicates onset and duration of stimulus. Dorsal roots C_4–T_8 and ventral roots C_8–T_{10} cut on the left side.

burning with the tip of a hot metal rod) caused no change in the resistance of the forepad of the same or the opposite limb. Flashing a light into the animal's eyes, blowing a whistle, or pinching any normally innervated skin, caused a sharp fall in the resistance of both forepads. Four cats with the left dorsal roots C_4 through T_2 cut were similarly examined, and all gave responses to stimulation of the region of the elbow and the posterior axillary fold of the affected limb.

These results may be given either of two interpretations: first, that the response depended on the presence of afferent nerves in the extremity being stimulated; second, that in the first group there was enough activity

in the sympathetic ganglia, which retained their normal preganglionic innervation, to mask any added reflex. In order to exclude this second and improbable possibility the preganglionic sympathetic paths to the deafferented forelimb were severed. The ventral roots of the left side were cut from T_1 through T_{10}. This deprived the limb of its preganglionic sympathetic innervation but left its sensory innervation intact and caused only a slight deficit of motor nerve supply. This resulted in an increase in resistance of the pad of the affected limb to 10,000–40,000 ohms above the normal level. The resistance was not altered by the most painful stimulation of sensitive areas of the cat's skin, and was therefore considered unaffected by the emotional state of the animal at the time of examination. When the stimulus was made excessively noxious, the cat could not be restrained for proper examination. Even when the stimulus was applied to the left forelimb no decline in resistance could be measured.

To avoid this difficulty and to test for reflex activity of the isolated ganglia under what was thought to be the most ideal of conditions, 3 cats were subjected to the following operation: the dorsal roots from C_4 through T_8 and the ventral roots from C_8 through T_{10} were cut on the left side. Thus, only the postganglionic sympathetic nerves of the limb were left, and not only were the sweat glands in the pad freed from impulses originating elsewhere in the body, but, since the limb was anesthetized, very strongly noxious stimuli could be applied without causing the cat any discomfort. No change in resistance could be produced by any kind of stimulation, even though pressure on the elbow was applied with forceps and large areas of skin on the arm crushed with hemostats. However, if leads were taken from the center pad of the opposite and normal limb, pinching the tail caused a fall in resistance of 2000 ohms, when the original resistance was 9000 ohms. The failure of the left forepad to respond cannot be attributed to the condition of the animal (fig. 2, record). It is attributed to the inability of the ganglia of the sympathetic chain to mediate reflexes when severed from their connections with the central nervous system.

Schwartz (4), however, came to the opposite conclusion. He measured the change in resistance in ten cats with "somatically-deafferented" right forelimbs (p. 597). Deep pressure on the deafferented limb caused a decrease in resistance of the left forepad. The following explanation was offered: "The mechanism causing this response probably consists of afferent sympathetic impulses passing down the right thoracic chain to below the level of the second thoracic; there the impulse can pass through the intact dorsal roots and cross over and upwards to the opposite side" (p. 598). In other words, Schwartz (4) explains a reflex initiated in deafferented tissue by suggesting that the tissue is not completely deafferented. One of the animals in his series was subjected to an additional

operation: the sympathetic chain above and below the right stellate ganglion was resected, and all rami from the ganglion were severed except the gray ramus to the first thoracic nerve. He concluded: "The right forelimb, accordingly, received its sole sympathetic supply from the stellate ganglion through one gray ramus, the ganglion being isolated from all other nervous connections" (p. 598). Reflex changes in resistance in this right forelimb (fig. 3, p. 598) were caused by auditory stimuli or by pinching the tail. This offers excellent evidence that the sweat glands of the right forelimb still retained a connection with the central nervous system. According to Nonidez and Hare (6), the gray rami may contain both pre- and postganglionic nerve fibers. Zuckerman (7) described a fusion of the white and gray rami in the monkey, and Kuntz (8) observed a similar arrangement in man. Langley (9) denied admixture of pre- and postganglionic fibers in the rami communicantes in the cat. In view of this anatomical evidence, it is dangerous to assume that no preganglionic fibers remain after section of all white rami to the sympathetic ganglion. A more dependable procedure for elimination of preganglionics is section of the appropriate ventral spinal roots as our third group of animals showed.

Bolton, Williams and Carmichael (10) studied the vasomotor responses in two patients with cord lesions. In one case the spinal cord caudal to the fifth thoracic segment was inactive, but the dorsal root ganglia and the sympathetic chain were preserved; in the other, a lesion of the cauda equina had destroyed the dorsal and ventral roots caudal to the second lumbar level. In neither case could vasomotor changes in the toes be elicited by stimulation of the anesthetized part of the body although the sympathetic chain ganglia were intact in each case.

Bronk et al. (11) divided a cardiac branch of the stellate ganglion of the cat into two fascicles. All other branches of the ganglion were then severed. Stimulating electrodes were applied to one fascicle of the cardiac nerve, recording electrodes to the other. Stimulation of one fascicle produced no response in the other. This is additional evidence against the idea of a reflex arc within the sympathetic ganglion.

Tonic activity in isolated sympathetic ganglia. Govaerts (12), and Tower and Richter (13) have presented evidence that sympathetic ganglia, when severed from the central nervous system, maintain a tonic or continuous activity, as distinguished from a reflex activity. Govaerts (12) recorded continuous discharges in postganglionic nerves arising from isolated sympathetic ganglia. Bronk et al. (11) failed to confirm Govaerts' findings. Tower and Richter (13) measured the galvanic resistance of the forepads of cats after section of the white rami, and later after removal of the thoracic sympathetic chain on the same side. The first of these operations was considered a preganglionic denervation, the second a postganglionic. Measurements of skin resistance begun before the first opera-

tion and continued after the second, showed that postganglionic denervation caused a greater rise in skin resistance than did section of the white rami to the ganglia. In other words, section of the white rami did not cause a total paralysis of the sympathetic ganglia. Their method of preganglionic denervation, section of the white rami, is subject to the criticisms made in the first part of this paper.

In order to avoid this source of error, we removed the thoracic and cervical sympathetic chain on the right side from six cats, and later cut the left ventral spinal roots from T_1 through T_9. This permitted simul-

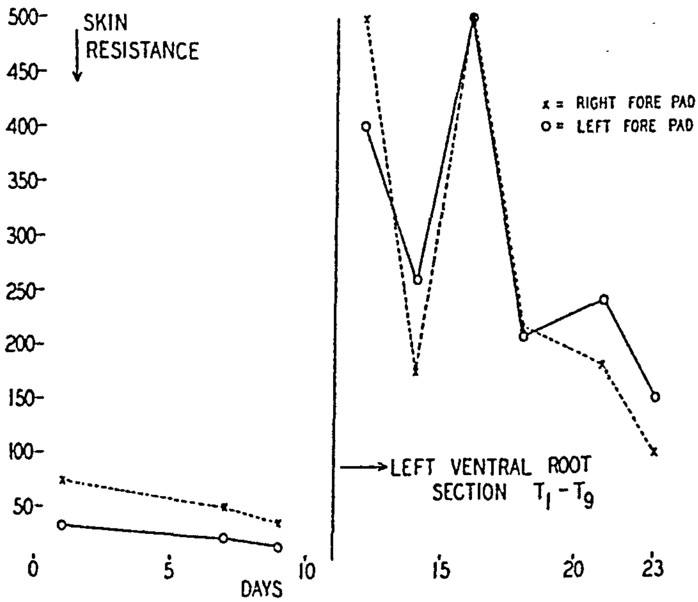

Fig. 3. Skin resistance of a cat after post- and preganglionic sympathectomy. Abscissa: time in days. Ordinate: skin resistance in thousands of ohms.

The right stellate ganglion and thoracic chain were excised two months before these observations were begun. Before the ventral root section on the 11th day, the resistance of the right pad was alwaysgreater than that of the left. After preganglionic denervation of the left side, this consistent difference disappeared.

taneous determinations of resistance from a pad deprived of its postganglionic nerves and from a pad without preganglionic innervation. The skin resistances of one of these cats is presented in figure 3. After removal of the right sympathetic chain from above the stellate through T_7, the resistance of the right forepad was consistently greater than the resistance of the left forepad. When the left ventral spinal roots from T_1–T_9 were cut, the resistance of both pads increased enormously. As the animal recovered, the resistances decreased together until the twelfth day after the ventral root section. At that time the animal was used for another experiment.

In similarly operated cats which were allowed to live longer no significant differences between the forepads could be detected until about four weeks after the ventral root section, when the regenerating fibers of the cut ventral roots began to establish functional connections with the postganglionic neurons, and the resistance of the left forelimb began to fall (14). In four to five weeks the resistance of the left forepad had returned to its pre-operative level and reflex changes could be elicited by pinching the tail. The high resistance of the right forepad persisted until the death of the animal.

SUMMARY

Since our experiments and those of others (10, 11) have failed to show activity in isolated sympathetic ganglia, and since it has been demonstrated that preganglionic denervation based on section of the white rami is incomplete, we feel that the preponderance of evidence supports the idea that the sympathetic ganglia of the upper thoracic chain, when severed from the central nervous system, are incapable of independent activity.

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THE EFFECT OF STEROIDS OF THE ADRENAL CORTEX AND OVARY ON CAPILLARY PERMEABILITY

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It has been demonstrated that the adrenal cortex has a significant relationship in the development of acute circulatory collapse. Some years ago it was shown that adrenalectomized animals were more easily put in a state of shock than normal ones by muscle trauma and other procedures, and that adrenal cortex extracts could ameliorate this lack of resistance to a considerable extent. More recently, it has been reported that desoxycorticosterone is effective in preventing the development of surgical shock and the peripheral circulatory failure of pneumonia, anesthesia, epinephrine and other agents. However, Swingle et al. (1) could not prevent the shock following intestinal stripping in adrenalectomized dogs by administering desoxycorticosterone acetate, confirming the results of Selye and Dosne (2) in intact rats, and of Weil et al. (3) in normal rabbits. Selye and Dosne have reported, however, that corticosterone administration was an effective measure for combating this type of shock.

A considerable amount of evidence has been presented which demonstrates that the adrenal cortex has an important function in regulating electrolyte and water metabolism of the body and it would appear that this property is involved in its relationship to peripheral circulatory failure. Darrow et al. (4) have postulated that the adrenal cortex regulates electrolyte metabolism by way of the kidney. On the other hand, Swingle et al. consider that the effect of the adrenal cortex in regulating body fluid is through the maintenance of the integrity of the peripheral vascular system.

The recent work of Menkin (5) is significant in this regard. He demonstrated that extracts of the adrenal cortex prevent or neutralize the effect of leukotaxin, a substance present in exudates which increases the permeability of skin capillaries. Menkin used adrenal cortex extracts which contained numerous steroids but he also indicated briefly that desoxycorticosterone had a similar effect. From these results it would appear that

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the rôle of the adrenal cortex in offsetting shock might be dependent on its ability to maintain capillary permeability.

Due to the fact, however, that the adrenal cortex extract and corticosterone behave somewhat differently from desoxycorticosterone in combating shock, it was decided to investigate the action of crystalline corticosterone and crystalline desoxycorticosterone acetate on capillary permeability. We have included in this study, the action of estrone, progesterone and stilbestrol since these possess some of the properties of cortical steroids in electrolyte metabolism.

Methods and results. Menkin's method of demonstrating changes in capillary permeability was followed with certain modifications. The various steroids used in our experiments were injected intracutaneously on the denuded abdomen of rabbits, alone and in combination with leukotaxin. Ten to twenty minutes later, 10 to 15 cc. of 1.5 per cent solution of trypan blue in physiological saline was injected intravenously and the concentration of dye at the sites of the intracutaneous injections was used as the index of change in capillary permeability. The crystalline steroids were water insoluble and since oil solutions were considered unsuitable for intracutaneous injection, these steroids were suspended in physiological saline or saline-gum acacia solution. The suspensions contained 1 to 5 mgm. of steroid per cubic centimeter and were fine enough to be drawn up into a 26 gauge needle.

Tests for the blank vehicles, i.e., saline and saline gum-acacia showed either no dye concentration or a blanched area. The following table indicates our results with adrenal cortex extract, corticosterone and desoxycorticosterone[2] on the permeability of the skin capillaries when injected by themselves and together with a solution containing leukotaxin. The local concentration of dye in the injected areas was graded by plus signs, i.e., "one plus" indicating a slight but definite concentration, "four plus," the maximum concentration. Doubtful concentration of dye is listed as "? plus."[3] Negative signs indicate similarly the degree of blanching, at the injected areas.

It can be seen from table 1 that the adrenal cortex extract neutralized the effect of leukotaxin in every instance and as a matter of fact, actually produced areas at the sites of injection which were paler than the untreated

[2] We are indebted to Dr. E. C. Kendall for crystalline corticosterone, Dr. V. Menkin for the preparation of leukotaxin, Dr. M. Gilbert (Schering Corp.) for crystalline desoxycorticosterone acetate and Dr. D. Klein (Wilson Laboratories) for adrenal cortex extract.

[3] The color due to dye concentration was unchanged following pressure with a glass slide on the affected skin, thereby ruling out the possibility that the color was the result of a dilatation of the local capillaries rather than an increase in capillary permeability.

skin. Corticosterone suspensions produced effects similar to adrenal cortex extract in preventing dye concentration. On the other hand, desoxycorticosterone was unable to neutralize the leukotaxin effect in every instance and actually induced a significant concentration of dye at times, when injected alone.

TABLE 1

*Effect of intracutaneous injections of adrenal cortex steroids on concentration of trypan blue**

	CORTICOSTERONE	CORTICOSTERONE WITH LEUKOTAXIN	DESOXYCORTICOSTERONE	DESOXYCORTICOSTERONE WITH LEUKOTAXIN	ADRENAL CORTEX EXTRACT	ADRENAL CORTEX EXTRACT WITH LEUKOTAXIN	LEUKOTAXIN
1	0	?+	0	++++	−−	0	+++
2	0	?+	0	+++	−	−	++
3	0	?+	+	+++	−−	−−	+++
4	0	0	++	+++	−−	−−	+
5	?+	0	+	+++	−−	−−	++
6	0		++		−		
7	0		0		−−		

* Desoxycorticosterone acetate was used. Each horizontal row represents results obtained in a rabbit during a single set of these experimental procedures.

TABLE 2

*Effect of intracutaneous injections of ovarian substances on concentration of trypan blue**

	ESTRONE	ESTRONE WITH LEUKOTAXIN	STILBESTROL	STILBESTROL WITH LEUKOTAXIN	PROGESTERONE	PROGESTERONE WITH LEUKOTAXIN	LEUKOTAXIN
1	+	+++	+	++	+++	++++	+++
2	0	+++	+	+++	+++	+++	0
3	+	++	0	++	++	+++	+++
4	0	+	+	+++	++	+++	0
5	+	++++	++	++	+++	+++	0
6	++	++	0	++	++	++	+
7	++	++++	+	+++	++	+++	0
8	0	++	++	+++	++	+++	+
9	+		++		++		

* Desoxycorticosterone acetate was used. Each horizontal row represents results obtained in a rabbit during a single set of these experimental procedures.

The following substances were similarly tested: estrone, stilbestrol and progesterone. Stilbestrol, though not a steroid, was included in order to determine whether its action on extra-gonadal tissues resembled that of estrone.

From table 2 it can be seen that these substances failed to neutralize leukotaxin, and at times induced a concentration of dye when injected

alone. Progesterone gave the most consistent effect in this regard. In several instances, both leukotaxin and the ovarian substances produced negligible dye concentrations when injected alone, but the combinations of leukotaxin with these substances resulted in a marked dye concentration.

The possibility was considered that the estrogens might liberate acetylcholine in the skin and thus produce their effects on the capillaries, since Reynolds (6) has indicated that estrogens increase the acetylcholine content of gonadal and extra-gonadal tissues. However, it was found that it required acetylcholine concentrations of 1 to 80 or even 1 to 20, to induce a moderate dye concentration. These amounts were often toxic or fatal to the test animals. Acetylcholine, therefore, appears to play no rôle in our results.

Discussion. It appears from our results that desoxycorticosterone, a substance which is concerned with salt and water metabolism, is unable to prevent an increase in capillary permeability under conditions where adrenal cortex extracts are effective. In addition, this substance may actually cause an increase in capillary permeability by itself. On the other hand, corticosterone which has relatively little effect on salt and water metabolism is quite capable of neutralizing the leukotaxin effect of increasing the permeability of skin capillaries. It is possible that the activity of adrenal cortex extracts in this respect is due to its content of corticosterone or similarly acting compounds.

From the above results it appears that the preventive action of desoxycorticosterone on shock following muscle trauma, anesthesia, epinephrine and intraperitoneal glucose is apparently not due to a maintenance of capillary permeability. It is more likely one of controlling blood volume through shifts in electrolyte content of body fluids. It is significant in this regard that Freed (7) was able to prevent the otherwise fatal shock of muscle trauma in adrenalectomized rats simply by administration of physiological saline. These results support the concept that shock following muscle trauma is primarily the result of hypohydremia. Of course, not all forms of shock are explained on this basis. Manipulation of the intestines produces a type of shock which cannot be prevented by desoxycorticosterone administration but can be prevented by adrenal cortex extracts or corticosterone (1–3). Thus, it would follow that in intestinal manipulation, changes in capillary permeability play a primary rôle.

In regard to the estrogen and progesterone effects, it appears that they simulate the action of desoxycorticosterone on the permeability of capillaries. It is noteworthy that the steroids able to maintain health and life in adrenalectomized animals, do not have identical effects on capillary permeability. It is likely, therefore, that the ability of steroids to maintain life following adrenalectomy is not necessarily related to their regulation of capillary permeability.

SUMMARY

1. Crystalline corticosterone, desoxycorticosterone and commercial adrenal cortex extract were tested for their effect on capillary permeability according to Menkin's "leukotaxin" method. Corticosterone as well as adrenal cortex extract prevented the action of leukotaxin in increasing the permeability of capillaries. Desoxycorticosterone did not do this but actually often produced a slight increase in capillary permeability by itself. These results may explain, in part, the different responses elicited by these preparations in protecting animals against the circulatory failure of various types of secondary shock.

2. Estrone, stilbestrol and progesterone were similarly tested. All of these substances not only failed to prevent the leukotaxin effect but produced an increase in capillary permeability when administered alone. It was demonstrated that these responses were not due to the local liberation of acetylcholine.

3. It is concluded that the ability of steroids to maintain the life of adrenalectomized animals is not necessarily related to their effects on capillary permeability.

We are indebted to Dr. L. N. Katz for his valuable suggestions in the course of this study.

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HEMOGLOBIN PRODUCTION INCREASES WITH SEVERITY OF ANEMIA[1]

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It is apparent from the experiments tabulated below that the hemoglobin output is stepped up as the anemia level of circulating hemoglobin sinks. This was not an unexpected reaction but we were surprised to find how closely the hemoglobin output was related to the intensity of the anemia stimulus in these standard dogs.

If we assume that the *stimulus* to hemoglobin production is *zero* in the normal dog which presents a circulating blood level of 21 grams hemoglobin per cent, we may safely say that the stimulus to hemoglobin production is *maximal* at an anemia level of about 6 grams hemoglobin per cent. This gives a maximal *anemia range* of 15 grams hemoglobin per cent in these dogs as used.

The *moderate anemia* level used in our experiments is 11 grams hemoglobin per cent or two-thirds of the anemia range of 15 grams hemoglobin per cent described above (21 grams − 11 grams = 10 grams hemoglobin per cent). The average values for hemoglobin production in moderate anemia are very close to two-thirds that found in the same standard dogs at the severe anemia level (table 1). These values obtain whether the dogs are tested with liver feeding or iron salts or liver extract given by mouth.

Stimuli which cause the body to produce blood proteins are not well understood—a magnificent understatement. Yet the adjustments in the normal dog are exquisite in their delicacy whether we consider the production of hemoglobin or fibrinogen or albumin of the blood. It is assumed generally that the stimulus of anoxemia is responsible for the production of new hemoglobin but there may be other stimuli related to this complex response (4).

Experimental observations. The general experimental procedure used in these standard anemia experiments has been reviewed in detail elsewhere (5). In general every effort is directed to the maintenance of uniform conditions.

[1] We are indebted to Eli Lilly and Company for aid in conducting this work.

Moderate anemia. Severely anemic dogs (hemoglobin 6 grams per cent) are permitted to attain a hemoglobin level of 11 grams per cent by the addition of the liver extract "Lextron" to the basal bread ration. Thereafter the dogs are fed the basal bread ration alone for several weeks until

TABLE 1

Hemoglobin production increases with severity of anemia

Figures represent net Hb production per 2 weeks

DOG NO.	LIVER FED*		IRON BY MOUTH†		LIVER EXTRACT FED‡	
	Anemia level					
	Moderate Hb—11 grams per cent	Severe Hb—6 grams per cent	Moderate Hb—11 grams per cent	Severe Hb—6 grams per cent	Moderate Hb—11 grams per cent	Severe Hb—6 grams per cent
	grams	*grams*	*grams*	*grams*	*grams*	*grams*
34-3	59 48	96 77 76	47 39	49 78 52	56	98
34-145	74 65	102 90	59 50	58 61	77	109 92
37-22	46 50	85 92 73	39	54 50 50 74 46	52	81 87
35-2	62 42	102 101 74 104 99	44 60	87 78 52 81 84 97	73	122 88 62 100
Average hemoglobin production.......	56	90	48	66	65	93

* Pig liver as fed averages 52 mgm. Fe per 300 grams daily.

† Iron by mouth = 40 mgm. Fe as amm. citrate.

‡ Liver extract as fed averages 270 mgm. Fe per day.

hemoglobin production is stabilized at a uniform base line level. The various diet factors are then tested under standard experimental conditions at the moderate anemia level. Other diet factors have been tested at this moderate anemia level but not in sufficient number to report. In general we may say that these experiments are in harmony with those tabulated below.

All dogs reported in table 1 were clinically normal, active, of uniform weight, with good appetite. In spite of this clinically normal state there is considerable variation in the response noted in repeat experiments on the same dog. Such variables are not rare in physiological experiments dealing with protein metabolism. In part they may be due to digestion factors, protein requirements other than new hemoglobin, and protein or iron stores in organs and tissues. Average values determined in several dogs from many repeat experiments we believe are significant.

Table 1 gives the *net hemoglobin production* over and above the control base line in each experiment. The four dogs used were well standardized and had been anemic under continuous observation for 4 to 6 years. During the greater part of this time the severe type of anemia had been maintained. The anemia level used in our published experiments (5) is 45 per cent of 13.8 grams hemoglobin or 6 grams hemoglobin per cent. This 6 grams hemoglobin per cent represents the optimum grade of severe anemia in these dogs. The dog can tolerate this degree of anemia without obvious clinical abnormality and loss of appetite. Below an anemia level of 5.5 grams hemoglobin per cent the dog may show clinical disturbance and the production of new hemoglobin may actually decrease. Obviously the anemia level should be kept as constant as possible by frequent bleedings with related blood volume determinations.

These dogs were raised in our own kennels from the strain used in all our anemia experiments and the normal hemoglobin level in these dogs is 20 to 21 grams hemoglobin per cent. They tolerate the customary regime very well and they continue a fairly uniform rate of hemoglobin production on various diets throughout a normal life cycle—often over 10 years continuously anemic.

The liver extract, 5.9 grams given daily ("Lextron"—Eli Lilly and Company) contains 270 mgm. Fe as fed. This extract (7) contains substances other than iron which have been shown to be potent for hemoglobin production in standardized anemic dogs. Cooked pig liver (300 grams fresh equivalent) as a standard test diet factor, is given daily for 2 weeks and all hemoglobin production is measured in grams per 2-week period.

It is to be noted that the responses to liver and liver extract are similar but the amount of contained iron very different. We have shown that factors other than iron in whole liver are responsible for some of the new formed hemoglobin. The group of food proteins represents one factor (2) but amino acids and related compounds (6, 1) may be at times responsible.

Discussion. When a *deficit* in circulating *hemoglobin* is produced (anemia) there is a relatively prompt response by the body (measured in days) with the production of new hemoglobin and red cells. It is obvious from these experiments (table 1) that the production of new hemoglobin

increases as the hemoglobin deficit (anemia) becomes more severe and there appears to be some parallelism between the hemoglobin output and the severity of the anemia. This is a relatively simple response as the new hemoglobin can be measured and hemoglobin cannot be put away in some large hidden reserve store.

When a *deficit* in circulating *plasma protein* is produced in the dog (hypoproteinemia) there is a complex response. There is a prompt (hours) inflow of needed plasma protein coming from reserve stores, also a more leisurely (days) appearance of new plasma protein coming from reserve stores and from food in the intestinal tract but this influx tends to bring the plasma protein levels back to normal. We believe that the plasma proteins and certain labile tissue proteins are in a fluid or dynamic equilibrium (3), which means that new plasma protein may move easily in or out of reserve stores held within tissue cells—a complex reaction which makes for uncertainty when one would measure the output of new plasma protein following plasma depletion. Nevertheless it is apparent that hypoproteinemia does stimulate new plasma protein accumulation within the circulating blood plasma.

When a *deficit* in *both hemoglobin and plasma protein* is produced simultaneously (4) we observe preference being given to hemoglobin production no matter what diet protein is utilized—a totally unexpected response which is worthy of much study. It seems probable that in some way the responses to anemia and hypoproteinemia are interrelated—that anoxemia is not the only stimulus concerned in the production of new hemoglobin.

SUMMARY

A severe anemia level of 6 grams hemoglobin per cent in dogs gives maximal stimulus for the production of new hemoglobin.

A moderate anemia level of 11 grams hemoglobin per cent under identical conditions gives a new hemoglobin production of approximately two-thirds of this maximum (table 1).

The term maximal *anemia range* is used to designate the difference between a normal blood of 21 grams hemoglobin per cent and a severe anemia of 6 grams hemoglobin per cent = 15 grams hemoglobin per cent. A moderate anemia (11 grams hemoglobin per cent) represents an anemia range of 10 grams (21 − 11 grams hemoglobin)—or two-thirds of the maximal anemia range.

The hemoglobin production therefore seems to run parallel to the degree of the anemia.

One stimulus to new hemoglobin production is believed to be anoxemia but there may well be other factors in this reaction.

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ESTIMATION BY THE FOREIGN-GAS METHOD OF THE NET (SYSTEMIC) CARDIAC OUTPUT IN CONDITIONS WHERE THERE IS RE-CIRCULATION THROUGH THE LUNGS

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In the course of studies on patients with patent ductus arteriosus (Keys, Violante and Shapiro, 1940; Shapiro and Keys, 1941) the estimation of the net (systemic) cardiac output became of interest. Eppinger, Burwell and Gross (1941) reflect the general belief that the foreign-gas methods cannot be applied to such conditions. We shall show that this belief is incorrect. The present paper is to present a general mathematical treatment for all conditions in which some fraction of the blood recirculates through the lungs and to give some results of the application of the relations which emerge from this theoretical analysis.

The considerations developed in this paper apply equally to nitrous oxide (Krogh and Lindhard, 1912), ethylene (Marshall and Grollman, 1928), ethyl iodide (Henderson and Haggard, 1925; Starr and Gamble, 1928), and to acetylene (Grollman, 1932). However, the acetylene method has been most widely used and is the method of choice in this laboratory, so the subsequent discussion will refer specifically to that method.

In the acetylene method Henry's Law applies, i.e., the amount of acetylene dissolved in the blood is proportional to the partial pressure of acetylene in the gaseous phase. Therefore, the amount of acetylene removed by the blood in unit time is directly proportional to the *effective* average partial pressure of acetylene in the lung-bag system and to the total blood flow through the lungs in unit time.

In the basic equation:

$$(1) \qquad \text{Cardiac output} = \frac{\text{oxygen consumption}}{\text{A.-V. oxygen difference}}.$$

The presence of a shunt of blood from the left to the right heart or equivalent does not affect the validity of the equation if we specify that the "cardiac output" refers to the net systemic output. Obviously, blood which has already been saturated with oxygen in the lungs and then re-enters the pulmonary circuit will not absorb more oxygen nor will it lose

any if the partial pressure of oxygen in the lungs is kept at 100 mm. or above. Such blood will not, in general, participate in the oxygen exchanges. With the foreign gas methods, however, the A.-V. oxygen difference is obtained from the concentrations of the foreign gas and hence this must be considered in detail.

Foreign-gas removal by the blood. The arterial-venous oxygen difference, which must be known in order to use equation (1) above, is equal to:

$$\text{A.-V. diff.} = \frac{(\Delta O_2)(C_{av})}{(\Delta C)Q}, \text{ (Grollman, 1932)} \tag{2}$$

where ΔO_2 is the difference in oxygen concentration between the first and second samples (corrected for the volume change of the lung-bag system), C_{av} is the average concentration of acetylene in the lung-bag system during the period between samples, ΔC is the acetylene difference between the samples (corrected for volume change) and Q is a constant for the solubility factor for acetylene in the blood at any given barometric prèssure and lung temperature. The cardiac output is, therefore:

$$V = \frac{(BMR)(\Delta C)Q}{(\Delta O_2)(C_{av})}. \tag{3}$$

In equation (3), C_{av} is the true effective average partial pressure of acetylene during the period when the acetylene concentration change, ΔC, was brought about. Now if C_a and C_b represent the concentrations of acetylene in the lung bag system in the samples taken at times t_a and t_b respectively, then the true average value C_{av}, may be calculated from the integral of $\frac{-dC}{dt} = k$, or

$$kt = \log_e\left(\frac{C_a}{C_b}\right). \quad \text{If } k' = \frac{k}{2.303}, \text{ then} \tag{4}$$

$$k't = \log_{10}\left(\frac{C_a}{C_b}\right). \tag{5}$$

Equations (4) and (5) represent, of course, the theoretical equation for a reaction of the first order; experimental verification of this equation is available and will be discussed later. Accordingly, the average acetylene concentration in passing from C_a to C_b is

$$C_{av} = \log^{-1}\left(\frac{\log C_a + \log C_b}{2}\right). \tag{6}$$

Now the *effective* acetylene concentration causing absorption of acetylene by the blood is the difference between the average concentration in the lung-bag system and that in the blood entering the lungs, or $\bar{C}_{av} =$

$C_{av} - C'_{av}$. If the blood entering the lungs is a mixture of ϕ parts of blood containing acetylene in equilibrium with C'_{av} concentration and $1 - \phi$ parts of blood containing no acetylene, then the *effective* average concentration will be $\bar{C}_{av} = C_{av} - \phi C'_{av}$ and the *total* cardiac output through the lungs will be

$$(7) \qquad V' = \frac{(BMR)(\Delta C)Q}{(\Delta O_2)(C_{av} - \phi C'_{av})}.$$

The evaluation of C'_{av} can readily be made in terms of the shunt-lung circulation time y and the total time t between t_a and t_b when the lung-bag samples are taken. It can be shown that:

$$(8) \qquad C'_{av} = C_{av}\left(\frac{C_a}{C_b}\right)^{y/t}.$$

Accordingly, equation (7) may be written:

$$(9) \qquad V' = \frac{(BMR)(\Delta C)Q}{(\Delta O_2)\left(C_{av} - C_{av}\phi\left(\frac{C_a}{C_b}\right)^{y/t}\right)},$$

and the true net output is

$$(10) \qquad V_0 = V'(1 - \phi) = \frac{(BMR)(\Delta C)(1 - \phi)Q}{(\Delta O_2)(C_{av})\left(1 - \phi\left(\frac{C_a}{C_b}\right)^{y/t}\right)}.$$

Now suppose we have attempted to calculate this output by the simple equation (3). The value we should obtain would be, as per cent of the true net output, 100 times the right hand term of equation (3) divided by the right hand term of equation (9), or:

$$(11) \qquad \text{Per cent true } V_0 = \frac{1 - \phi\left(\frac{C_a}{C_b}\right)^{y/t}}{1 - \phi} \times 100.$$

It will be noted from equation (10) that the true net (systemic) cardiac output will be underestimated if the simple equation (3) is used when there is a re-circulation because the ratio C_a/C_b must always be greater than 1.0 and hence the entire expression $\left(\frac{C_a}{C_b}\right)^{y/t}$ will also be greater than 1.0 no matter what may be the values of y and t. Furthermore, y/t will normally be only a fraction of 1.0 and $C_a - C_b$ is ordinarily small compared to C_a, i.e., C_a/C_b will be considerably less than 2.0, so the entire expression $\left(\frac{C_a}{C_b}\right)^{y/t}$ cannot be *much* greater than 1.0 and we may expect the error from the use of equation (3) to be small.

The magnitude of the error may be calculated for a sample case. The sample data given by Grollman (1932, p. 66) may be used. Here the corrected acetylene concentrations are $C_a = 10.76$, $C_b = 9.36$. We shall indicate later that the lung-circulation time, y, is of the order of 3 to 6 seconds. If we assume that $y = 5$ and that the time between samples is $t = 10$, we may calculate the effect of a "leak" or re-circulation of 40 per cent, that is, where 40 per cent of the blood ejected from the left ventricle passes through the ductus and makes a second transit of the lungs. In this case, then, the net cardiac output calculated by the ordinary equation (3) would be, as per cent of the true net output:

$$\text{Per cent } V_0 = \frac{1 - 0.4\left(\frac{10.76}{9.36}\right)^{5/10}}{1 - 0.4} \times 100 = 95.2,$$

and the error would be only -4.8 per cent.

When the ordinary calculation is made in cases with re-circulation the net output will be under-estimated by an amount dependent on the 3 factors, C_a/C_b, y/t and ϕ. We may consider the magnitudes of these factors individually.

The ratio C_a/C_b. In order to show what we may expect for values of C_a/C_b we have analyzed all the data in the last 2 notebooks of this laboratory, covering 89 consecutive acetylene experiments, 40 on patients with patent ductus arteriosus and 49 on normals and patients with heart disease not involving re-circulation. In this series C_a/C_b averaged 1.23, $\sigma = \pm 0.116$ in the patent ductus arteriosus patients and 1.25, $\sigma = \pm 0.085$ in the others. The extreme range was 1.07 to 1.44 with the exceptions of 2 experiments of dubious technical validity where the apparent ratio C_a/C_b was 1.47 and 1.57.[1] The person on whom the highest ratio, 1.57, was found, has since been studied on 3 occasions where we found the ratios 1.10, 1.18 and 1.28.

The ratio y/t. The value of t, the time between the gas samples, is ordinarily of the order of 10 to 15 seconds, but the proper timing has been the subject of some discussion (cf. e.g., Starr and Collins, 1933; Gladstone, 1935; Adams and Sandiford, 1941). It seems agreed that adequate mixing in the lung-bag system requires no more than about 6 to 8 seconds with proper breathing (op. cit.) and that has been our experience. Grollman (1932) reported that evidence of recirculation of systemic blood normally never appears before 23 seconds from the start of rebreathing and therefore the second gas sample can be taken at that time. Careful studies on a small number of persons in this laboratory indicate that an even longer

[1] These highest values, though questionable, were included in the averages cited above.

time, at least 26 seconds, may be used in subjects at rest. In normal subjects, then, t may be as long as 18 or 20 seconds.

Where there is a short-circuit re-circulation through the lungs, however, the first gas sample should not be taken before blood in equilibrium in the lungs begins its recirculation via the short circuit. In other words, mixing in the lungs should be completed at least y seconds before the first sample is taken. We must consider the lung-circuit time, y, before deciding on the proper duration of t.

The volume of blood in the lungs at any one time represents about 7 per cent of the total blood in the body (Spehl, 1883; Menicanti, 1894; Tigerstedt, 1903). From this we can calculate that, in rest, the lung-circuit time would normally be about 4 seconds. Starr and Collins (1933) found the lung-circuit time to be "about 5 seconds" in direct experiments. Where there is a shunt involving re-circulation the lung vessels may be somewhat engorged (Keys, Violante and Shapiro, 1940) but the velocity of the total blood flow is, of course, higher than normal so that the lung-circuit time in these cases may even be less than in normal persons.

From the foregoing, we conclude that the first gas sample should be taken at around 13 seconds and if the second sample is taken at 25 seconds the ratio y/t is about 0.3 and even when the second sample is taken at 23 seconds the ratio y/t should not exceed 0.5 in the absence of heart failure and pulmonary congestion.

The value of ϕ. The magnitude of the re-circulation, ϕ, in patent ductus arteriosus has been estimated in 3 cases by Eppinger, Burwell and Gross (1941) from oxygen analyses for blood taken at various sites and times during operation to close the ductus. In spite of the difficulties of proper sampling and the abnormal conditions—anesthesia and the chest open—the values calculated are of interest. In 2 cases it was suggested that about 50 per cent of the left ventricular output passed through the ductus; in the third case a value of 77 per cent was obtained. In the latter case the calculated left ventricular output seems impossibly high—25 liters per minute in a girl of 42 kgm. body weight with only "moderate cardiac enlargement." The general conclusion from these studies would be that ϕ may be around 0.5 in severe cases of patent ductus arteriosus.

We have studied 27 cases of patent ductus arteriosus with our simultaneous roentgenkymography and acetylene re-breathing methods (Keys et al., 1939, 1940). Repeated studies were made on many of these patients and the results were satisfactory in 22 of them. Even without correction for the re-circulation effect the comparison of the stroke-volume change of the heart with the acetylene removal from the lungs permits an approximate estimate of the value of ϕ. These (uncorrected) values for ϕ ranged up to 0.7 in several very severe cases and were of the order of 0.2 to 0.4 in the majority of the patients.

The range of values of ϕ in interventricular septal defects is entirely conjectural at present. In general, we may expect that the shunt of blood from left to right heart should be of the same order of magnitude as in patent ductus arteriosus.

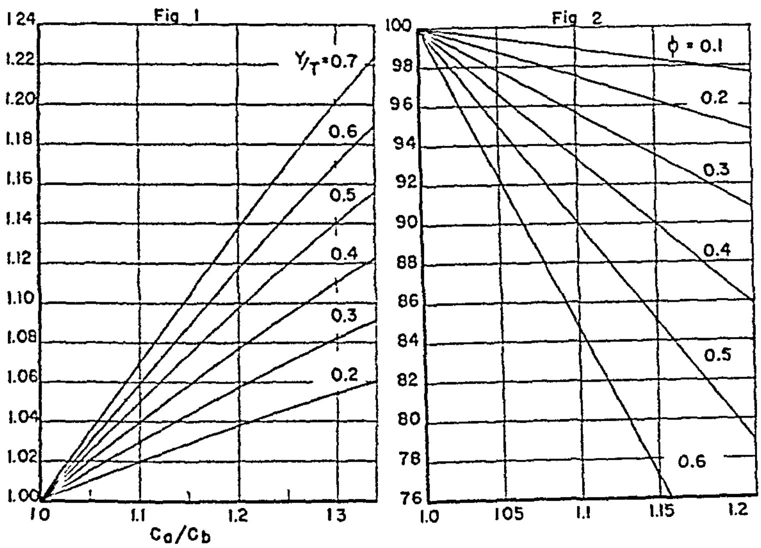


Fig. 1. Values, on the rectangular ordinate, for the expression

$$\left(\frac{C_a}{C_b}\right)^{y/t}$$

for various values of C_a/C_b and for y/t.

Fig. 2. Values for

$$\cdot 100 \left(\frac{1 - \phi \left(\frac{C_a}{C_b}\right)^{y/t}}{1 - \phi}\right),$$

on the rectangular ordinate, for various values of ϕ and for

$$\left(\frac{C_a}{C_b}\right)^{y/t}$$

(on the rectangular abscissa).

The evaluation of equation (11). In order to illustrate the magnitude fo the total error and to facilitate calculations for particular cases, we have prepared three-dimensional graphs. Figure 1 gives values for $\left(\frac{C_a}{C_b}\right)^{y/t}$ corresponding to values of $\left(\frac{C_a}{C_b}\right)$ up to 1.35 and y/t up to 0.7. Figure 2 gives the values for the uncorrected cardiac output as per cent of the true net output corresponding to recirculation up to 60 per cent and values of $\left(\frac{C_a}{C_b}\right)^{y/t}$ up to 1.22.

We have been able to use equation (11) to estimate satisfactorily the errors resulting from uncorrected application of the acetylene method in 22 patients with patent ductus arteriosus. The maximum error was −12 per cent in an experiment with patient R. T., where ϕ was about 0.6, C_a/C_b was 1.21 and y/t was about 0.4. In other words, the uncorrected calculation gave a result 12 per cent less than the true net systemic output of the heart. The average error was −3.36 per cent, $\sigma = \pm 3.19$ per cent for 41 separate experiments with the 22 patients.

The reaction order for foreign-gas absorption. We have already stated that the absorption of acetylene proceeds as a first order reaction. Theory demands this monomolecular reaction behavior and it is confirmed by the fact that a straight line is obtained when the logarithm of the acetylene content (or concentration corrected for volume change) in the lung-bag system is plotted against time (Gladstone, 1935). We have confirmed this result but such a test requires a very well trained subject and care in taking the gas samples at precisely the same phase of respiration. The test is much more simply made by plotting the acetylene removal against the oxygen removal.

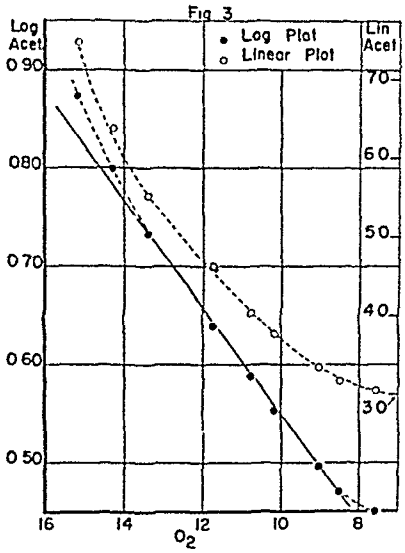
If the blood flow and metabolism are constant, the removal of oxygen from the lung-bag system proceeds linearly with time so long as the oxygen partial pressure is not allowed to fall below the point where the hemoglobin is practically saturated with oxygen in the lungs. Accordingly the indication that the acetylene absorption conforms to an equation of the first order is obtained if the plot of the oxygen content in the lung-bag system against the logarithm of the acetylene content conforms to a straight line. For "contents" the concentrations, corrected for volume change may be used, of course.

Figure 3 reproduces the results of a typical experiment in which 9 successive gas samples were taken during rebreathing. Note that in such experiments with multiple sampling the volume change resulting from the withdrawal of the samples must be allowed for in calculating the contents or corrected concentrations of the lung-bag system. This point has been generally disregarded by investigators using multiple sample procedures.

It is clear that the acetylene removal conforms closely to the expectation of a first order equation and that the rate of acetylene removal is constantly proportional to the acetylene concentration from the time equilibrium is established (sample 3) for 6 samples, a period of 27.7 seconds in this case. Similar results have been obtained with all other subjects tested, but the period of constant removal rate is usually somewhat shorter, averaging about 22 seconds in rest.

Experiments like that depicted in figure 3 verify equations (4) and (5) above and show that the final gas sample can be taken later than is currently believed possible. The reason for the long "safe" period for

sampling is undoubtedly that the first blood which carries acetylene in the systemic circulation loses most of this acetylene in equilibrating with the tissues before it returns to the heart (cf. Starr and Collins, 1933).



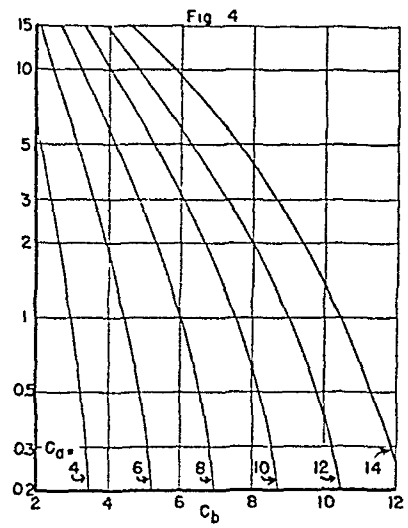


Fig. 3. Results of a typical acetylene re-breathing experiment in which 9 successive gas samples were taken. The acetylene and oxygen values in these samples have been corrected for the changes in the volume lung-bag system resulting from gas absorption in the lungs and from the withdrawal of the gas samples. Acetylene values on the ordinates, logarithmic scale on the left, linear on the right.

Fig. 4. Values for $-E$ in equation (14) on page 276, for various values of C_a and C_b. E is the error, in per cent of the true value, resulting from the use of the ordinary Grollman calculation for the average acetylene concentration in the lung-bag system between samples.

Average acetylene concentration. It is obvious that the average acetylene concentration should be calculated according to equation (6) above. Conversely, the ordinary Grollman calculation:

$$C''_{av} = \frac{C_a + C_b}{2} \neq C_{av}. \tag{12}$$

It is important, therefore, to discover the magnitude of the error resulting from the use of equation (12) rather than equation (6). It is readily shown that the error resulting if the Grollman equation (12) is used would be, as per cent of the true output:

$$E = 100\left(1 - \frac{V''}{V_0}\right) = 100\left(1 - \frac{1/2(C_a + C_b)}{\log^{-1} 1/2(\log C_a + \log C_b)}\right). \tag{13}$$

It will be noted that E must always be negative since the ratio of C''_{av} to C_{av} will always exceed 1.0; i.e., the ordinary Grollman calculation will

always over-estimate the true average acetylene concentration and accordingly under-estimate the cardiac output. For this reason and to expedite calculation, equation (13) is more conveniently used in the form:

$$(14)\quad -E = \log^{-1} (2 \log \tfrac{1}{2}(C_a + C_b) - \tfrac{1}{2}(\log C_a + \log C_b) - 100.$$

Application of equations (13) or (14) to specific cases shows that the error is generally small and frequently entirely negligible. For example, E in the sample case from Grollman (1932, p. 66) is only -0.18 per cent. In some instances, however, it may be of consequence. Evaluation may be made from figure 4 in which we have plotted values for E for all values of C_a and C_b likely to be encountered in combination.

Oxygen exchanges. The negligible rôle of oxygen exchanges in cases of recirculation has already been mentioned. This is not true if the oxygen partial pressure falls below the level at which the hemoglobin of the blood is effectively saturated in the lungs. At body temperature and a partial pressure of CO_2 = 40 to 50 mm., human blood is about 96 per cent saturated with oxygen at 80 mm. Hg and 94 per cent at 70 mm. This means that, if the barometric pressure is 760 mm. the oxygen concentration in the lung-bag system would have to fall to around 10 or 11 per cent to produce a slight reduction in the rate at which oxygen is removed by the blood hemoglobin. It should be remembered that the oxygen gradient between alveolar air and arterial blood is only 1 or 2 mm. when the oxygen partial pressure in the lungs is of the order of 70 or 80 mm. (cf. e.g., Keys, 1938, p. 610).

The effect of changing partial pressure of oxygen in the lung-bag system on the oxygen removed in simple physical solution in the water of the blood is, of course, directly proportional to the oxygen partial pressure in the blood. If the arterial oxygen saturation falls as low as 90 per cent at the time of the second gas sample this would mean that the physically dissolved oxygen would only be about 0.04 volume per cent less than at the start. Since the total arterial-venous oxygen difference is of the order of 6.0 volumes per cent the maximum error from changing oxygen in physical solution is only about 0.7 per cent.

Coronary circulation. If the re-circulation involved tissues which themselves use up a large amount of oxygen from the blood these arguments would not apply. Such might be argued for the special case of the coronary circulation where the situation is complicated for both oxygen and acetylene or other foreign gas. Hamilton, Spradlin and Saam (1932) and Gladstone (1935) have suggested that the return of blood from the nearest circuits, especially the coronaries, constitutes a re-circulation that begins so quickly after the start of re-breathing that the whole experimental period should be made very short—less than 15 seconds.

A detailed analysis of the coronary re-circulation effect would be too

lengthy for inclusion here but we may state that the oxygen usage by the heart itself is a part of the total metabolism and so its effect on the oxygen exchanges need not be specially considered. Blood going through this system would, however, return rapidly to the lungs and would tend to alter the acetylene exchanges on re-entry. Two points may be noted.

In the first place, the heart itself would absorb a considerable part of the acetylene contained in the blood making its first circuit through it and would tend to equilibrate with it with further portions of blood. The solubility of acetylene in muscle is not greatly inferior to that in blood and may even exceed it if much fat is present. In the second place, the coronary blood flow is only of the order of 10 per cent of the total cardiac output. Equations (10) and (11) apply and ϕ is about 0.1. It does not seem possible for the coronary flow to introduce any considerable or even appreciable error. Even if y/t should be as large as 0.7 or 0.8 the resulting error is only of the order of 2 per cent.

The cardiac index in patent ductus arteriosus. In all we have made 41 satisfactory measurements of the net cardiac output under basal resting conditions in 22 patients with simple patent ductus arteriosus. None of these patients was in failure. By "satisfactory" is meant that all technical details were unexceptional, duplicate analyses agreed and adequate bases were available to estimate the approximate correction for the re-circulation effect. This last averaged 3.36 per cent, with a maximum of 12 per cent in one patient. The experiments were all made in a quiet room at 78°F. Body surface was computed from the height and weight by the charts of DuBois (1936).

The cardiac index, liters of blood per square meter of body surface per minute, averaged 2.45, $\sigma = \pm 0.520$, minimum 1.44, maximum 3.77, in this series. The average minimum for any one patient was 1.55, and the average maximum was 3.28. In 4 patients the cardiac index averaged less than 2.0. The range of these values is greater than and the average is slightly higher than for the large series of normal subjects studied in this laboratory. It is notable that the higher values tended to occur in patients with elevated metabolism.

The patients in this group averaged 15.7 years in age, ranging from 5 to 35 years old. We are unaware of any acceptable standards for cardiac index on a group of normals of comparable age but all indications are that the basal cardiac index is not much different in a group of this age composition from that found in young adults. The average cardiac index for normal young adults in this laboratory is about 2.4 under the same conditions used with the patients.

It is clear that the average cardiac index in our series of patients with patent ductus arteriosus is either normal or close to it. In 4 patients (16.7 per cent) the index is definitely subnormal, but in 5 patients (20.8

per cent) the index seems to be above the normal expectation. This is in full agreement with our results with patients with compensated heart disease of other types.

Discussion. The analyses and arguments advanced here apply only to conditions where the timing of the gas samples is reasonably correct. The first sample should not be withdrawn before blood from the short circuit, containing acetylene in equilibrium with the mixed gas, has made a re-entry into the lungs. The requirement that the second sample be withdrawn before the rate of acetylene absorption in the lungs changes is not likely to be unfulfilled in rest, as pointed out above.

We may inquire what would be the result if the first sample is withdrawn too early, say after mixing has taken place but before blood in equilibrium with the mixed gas has been able to re-enter the lungs. For example, suppose mixing is complete in 8 seconds, the lung-circuit time is 4 seconds and the first sample is taken at 10 seconds and the second at 22 seconds. The worst imaginable condition would be where for 2 seconds re-circulated blood containing *no* acetylene would be entering the lungs where it would, of course, absorb acetylene just like the true venous blood. This would be the equivalent of a relative blood flow of $1 + \phi$ instead of 1 for $\frac{1}{6}$ the period of measurement. If ϕ were 0.5 the result would be that the net circulation would be over-estimated by about 10 per cent.

Actually, during the initial 2 seconds of the period between samples the re-circulated blood entering the lungs would have far more than zero acetylene concentration. The proper expectation may be gauged from measurements of the rate of change of acetylene concentration in the lung-bag system during 3 or 4 seconds before mixing is complete. Experiments on this point indicate that in this period the alveolar air is less concentrated in acetylene than the bag gas but that if complete mixing takes 8 seconds the concentration in the alveolar air is not less than 80 per cent of the concentration in the bag gas at 6 seconds. Accordingly the error in the case cited would only be an overestimate of the order of 1 per cent.

It is obvious that the danger of error of the type just discussed is slight. Here, as elsewhere in this paper, the conclusions with regard to timing refer only to resting conditions and do not apply to exercising subjects.

Many arguments and suggestions have been made in the literature as to large and difficultly controlled sources of error in the foreign-gas methods for the estimation of the cardiac output. We may divide these into 2 groups, those having to do with standardization of physiological conditions and excitement, and those having to do with re-circulation, mixing, analysis and computation. The present paper has to do with the latter group of potential sources of error. It seems that these are nothing like so serious as is frequently supposed and, in general, they may be almost completely

eliminated if attention is paid to the lessons of considerations like those developed here.

On the other hand we are convinced that the difficulty of physiological standardization is generally underestimated and this applies not only to the foreign-gas methods but to all other methods for circulation measurement. In our experience it is more difficult to attain reproducible cardiac output standardization than metabolic standardization and the environmental and psychological conditions must be more rigorously controlled than for basal metabolism measurements. We deplore, therefore, the fact that this point is often neglected and this to an increasing extent as estimations of cardiac output are extended to a larger number of laboratories and hospitals.

SUMMARY

Some fundamental kinetics have been analyzed for the gas absorption by the blood in the foreign-gas methods for estimation of the cardiac output in man. The discussion applies specifically to the acetylene method but the conclusions apply to the other foreign gases.

It is shown that the absorption proceeds according to an equation of the first order and the mathematical analysis is developed accordingly.

The condition where re-circulation occurs in the lungs is analyzed in detail and equations are derived for the proper computation of the true systemic circulation in such cases. The variables involved are the concentrations of the foreign gas in the gas samples from the lung-bag system, the fraction of blood re-circulated, the short-circuit time, and the total time between samples. Graphs for computations with these variables are presented.

It is shown that re-circulation through the lungs in conditions such as in patent ductus arteriosus or in inter-ventricular septal defects does not necessarily introduce a serious error and that this error may be estimated.

It is shown that re-circulation of blood from the coronary system cannot introduce an appreciable error.

The error resulting from the common assumption that the absorption of the foreign-gas is linear with time is discussed and shown to be ordinarily small. Means are provided to estimate this error by equations and a graph.

Timing of the gas sampling is discussed. It is shown that the second sample may be taken later than is frequently believed possible. When there is re-circulation the first sample should be delayed 4 or 5 seconds but it is shown that 2 or 3 seconds are not critical.

Results of 41 studies on 22 patients with patent ductus arteriosus are presented in summary form. The average cardiac index is normal or close to it in this group.

It is indicated that many criticisms of the foreign-gas methods are in-

valid because they are based on quantitative misconceptions. On the other hand the importance and difficulty of physiological standardization in cardiac output measurements are frequently under-estimated.

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TOLERANCE OF THE NEWBORN TO ANOXIA[1]

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From many points of view it is important to make a comparative study of the tolerance of the newborn and adult animal to oxygen lack whether produced by partial or complete replacement of oxygen of the inspired air by nitrogen, or by respiration of pure helium, nitrous oxide, cyclopropane, or carbon dioxide. These studies were not limited to the newborn but also include animals at various ages postpartum as well as fetuses in utero. Kabat (1) has already demonstrated the comparatively longer survival time of the newborn during asphyxia.

METHOD. Adults and infants of the following species were studied: rat, rabbit, cat, dog and guinea pig. Each animal was placed in a jar so arranged that its contents could be rapidly replaced by any desired gas. In some experiments the temperature of the jar and that of the animal within were recorded. The criterion used to indicate survival was the persistence of respiratory efforts, the end point being the time when these movements could no longer be evoked. The effect of the injection of 5 mgm. of sodium cyanide was studied both in young and adult animals breathing air. Estimations of the blood sugar level, by the Hagedorn and Jensen method (2), were made on samples of blood collected from rats and puppies before and during anoxia. The blood obtained from the puppies was also analyzed for oxygen contents by the method of Van Slyke and Neill (3). Electrocardiograms were recorded from these puppies. In order to estimate the influence of the brain on the length of the survival period, cerebral tissues were excised from rats of various ages and the oxygen consumption was determined in the Warburg apparatus. Pregnant animals respired mixtures of 5 per cent oxygen in nitrous oxide until exitus. The fetuses were then removed by Cesarean section and their behavior observed. Some of the pregnant animals were given lethal doses of sodium cyanide. After the mother succumbed, the fetuses were studied.

RESULTS. The adult rat exposed to an atmosphere of pure nitrogen undergoes a short period of excitement, becomes comatose, and succumbs after approximately 1.5 minutes. Figure 1 demonstrates that rats 1 day

[1] Aided by a grant from the Child Neurology Research (Friedsam Foundation).

of age survive for about 50 minutes at an environmental temperature of approximately 24°C. The points on the curve represent averages of numerous observations. As the rat progresses in age his tolerance to anoxia decreases and at approximately 17 days of age the sensitivity is like that of the adult. The oxygen consumption of minced cerebral tissue of infant rats of various ages reveals the following. From 1 to 10 days there are no significant changes. The cerebral metabolic rate, however, is well below that of the adult (4, 5). After 10 days of age there is a rapid increase of the oxygen uptake of the infant rat brain.

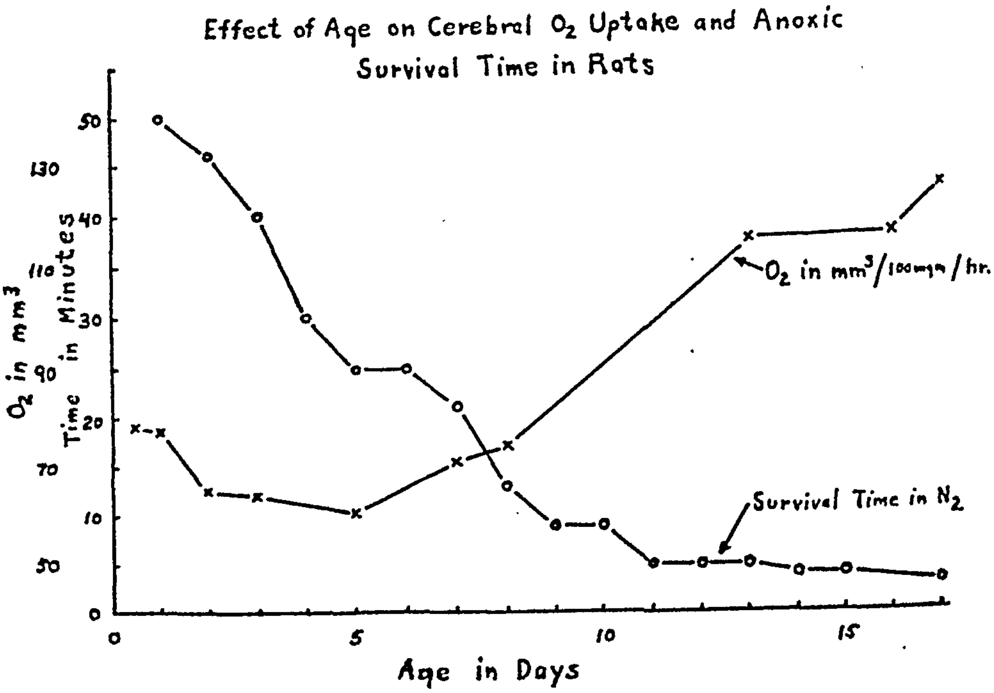

Fig. 1

The temperature of the newborn which approximates that of his environment has an important influence on the survival period. In a raised environmental temperature the survival period is decreased. At 1, 2, 4, 6 and 7 days of age the average survival periods were reduced and varied from 21 to 8 minutes as a result the environmental temperature being increased from 24 to 34°C. At either temperature the newborn far outlasts the adult.

Studies made on 26 newborn dogs reveal a survival period in nitrogen which may be extended to 43 minutes for puppies 1 day of age who are members of a vigorous litter. This period becomes shorter during the first week of life and in 1 observation of an animal 1 month of age, the time was reduced to 13 minutes. Analysis of repeated samples of arterial blood discloses that it contains practically no oxygen within the error of the

method after several minutes of nitrogen inhalation. The blood sugar demonstrates the asphyxial rise. Table 1 presents the survival period, blood glucose, and oxygen contents of some of the puppies studied. Among the newborn of other species it has been observed that on the average 5 kittens respired from 20 to 30 minutes, 5 rabbits for about 17 minutes, and 6 guinea pigs only 7 minutes in an atmosphere of nitrogen.

The same differential effect produced by nitrogen can also be demonstrated by the use of sodium cyanide. Concentrations which are lethal

TABLE 1

Survival periods of infant dogs respiring nitrogen

AGE	PERIOD IN NITROGEN	CONDITION AT TIME OF REMOVAL*	TIME OF BLOOD COLLECTION	OXYGEN CONTENTS ART. BLOOD	BLOOD SUGAR
days	*minutes*		*minutes*	*volume per cent*	*mgm. per cent*
1	16	D	14	0.23	
1	20	A†			
1	20	D	8	0.71	209
1	22	A	22	0.02	
1	29	A	13	0.02	
1	33	A	33	0.00	
1	39	A	39	0.00	
1	43	D	43	0.60	
2	24	D	4	0.36	
2	25	A	25	0.72	
4	22	A			
5	8	A			
6	28	D	5	0.04	104
			11	0.12	354
			16	0.05	336
			21	0.05	394
7	17	A	17	0.07	
13	10	A	3	0.71	131
			9		343
30	13	D			

* D = dead; A = alive.

† After 2 months animal in good condition.

to adult rats in 5 minutes permit infants to make respiratory efforts for an average of 61 minutes. It is interesting that the replacement of oxygen with nitrous oxide presents results exactly comparable with those of nitrogen. Rats 1 day of age survive for approximately 48 minutes. This duration decreases with age. At 1, 2, 3, 6 and 8 days the period of tolerance averages 52, 43, 36, 21 and 13 minutes respectively. Experiments on newborn rats in helium disclose results similar to those observed with nitrogen and nitrous oxide. Rats and dogs subjected to 5 per cent oxygen in nitrous oxide far outlast those respiring pure nitrous oxide. The adult

rat lives for 14 to 20 minutes and the infant usually at least 12 hours. Two adult dogs survived for about 15 minutes while 4 infants in the same mixture lived from 50 to 200 minutes. Baby rats do not tolerate carbon dioxide and cyclopropane as well as nitrous oxide but far outlive adults exposed to the same mixtures. The survival times with pure carbon dioxide at 1, 2, 3, 4, 5, and 10 and 12 days of age are 23, 28, 13, 13, 7 and 5 minutes respectively (25 observations). Twenty-four 1 day old rats lived in cyclopropane from 13 to 35 minutes. Sixteen newborn rats submerged in water at 37°C. continue to make respiratory movements for long periods. They survive at least 40 minutes, recover, and apparently develop normally. When the water contained india ink, granules of that substance

TABLE 2

Survival periods of newborns of different species subjected to various procedures

NUMBER OF OBSERVATIONS	AVERAGE SURVIVAL PERIOD	AGENT	SPECIES
	minutes		
150	50	Nitrogen	Rat
5	25	Nitrogen	Cat
18	23	Nitrogen	Dog
5	17	Nitrogen	Rabbit
6	7	Nitrogen	Guinea pig
17	20	Nitrogen (32–35°C.)	Rat
15	48	Nitrous oxide	Rat
11	26	Carbon dioxide	Rat
24	24	Cyclopropane	Rat
16	40	Submersion in water	Rat
9	61	Sodium cyanide	Rat
6	52	Helium	Rat

could be detected in the lungs of those animals that were sacrificed. The above results on the newborns are summarized in table 2.

Five pregnant animals, 1 cat and 4 rats were subjected to a mixture of 5 per cent oxygen in nitrous oxide until exitus. The adult cat succumbed after 10 minutes but all her 4 fetuses respired spontaneously on removal and survived until sacrificed. Similar results were observed on the rats. Despite the anoxic death of the mother and the fact that the fetuses were permitted to remain in utero 5 minutes thereafter some of them were successfully raised by foster mothers. Fetuses breathing spontaneously were delivered from pregnant rats given a lethal concentration of sodium cyanide. These infants survived for varying periods.

Four to 5 day old infant dogs which breathed pure nitrogen for 7 to 10 minutes showed the following electrocardiogram changes: first sinus arrhythmia, then slowing of rate succeeded by disappearance of the P wave within the first two minutes. Vagal dominance is probably indicated

during this period. Ventricular escape follows with nodal beats, idioventricular rhythm at a rate of 35 to 50 per minute. Then, possibly due to a diminution of the vagospasm, the P wave returns and after 5 minutes of anoxia, the rate becomes more regular and faster than after the first 2 or 3 minutes. If the anoxia is still further prolonged, heart action gradually weakens and cardiac standstill may be produced.

In one puppy there was a slight elevation of the ST segment. Ectopic beats, auricular fibrillation and flutter were seen in another puppy after 8 minutes. In different puppies SA node block, prolonged AV interval, occasional AV block, and bundle branch block were seen. Generally, with cessation of the anoxia the original rates returned with only slight irregularities. The changes in the electrocardiogram are essentially similar to those observed in adults but require more profound anoxia for development. It is significant that the heart continues to beat long after it is possible to evoke respiratory effects.

Discussion. From these results it may be concluded that under a wide variety of conditions the newborn exhibit a greater tolerance to anoxia than the mature of the same species. Resistance, however, does vary among species and seems to depend upon the degree of physiological maturity at the time of birth. For example, the newborn rat, which survives for approximately 1 hour, is without hair or teeth, has unopened eyes, is totally dependent on its mother and acts like a bulbo-spinal animal. The newborn guinea pig, on the other hand, which lives in an atmosphere of pure nitrogen for only 7 minutes is a comparatively mature animal exhibiting coördinated locomotion, righting reflexes, temperature regulation and therefore a functioning cephalad portion of the brain stem. In all species there is a loss of tolerance at a rate which seems to depend upon post natal development. For example, the rapidly developing rat at 18 days of age exhibits the diminished adult tolerance while the more slowly maturing dog is more resistant than the adult at 30 days of age. Kabat (1), who studied the resistance of the canine brain to cerebral anemia, observed that the tolerance of the young dog gradually decreases until 100 days of age, at which time it is indistinguishable from the adult. At this point it is interesting to compare the ages at which the oxygen consumption of excised brain of rat and dog attain the higher values of the mature animal. In the rat at 24 days of age the oxygen consumption of the brain is like that of the adult (4) while the higher oxygen uptake of the mature dog is developed in the infant when it attains the age of 35 to 50 days (6).

Since sodium cyanide inhibits practically all tissue respiration by inactivating the heavy metal carrying respiratory pigments, it might have been expected that the response to sodium cyanide would be similar to that with nitrogen. Nitrogen acts by displacing oxygen while cyanide prevents the utilization of that gas.

Despite a specific narcotic action of nitrous oxide the survival period of

the infant rat in that gas was similar to nitrogen. Perhaps the lack of the development of the most cephalad portion of the neuraxis, the part of the brain where nitrous oxide may exert its primary narcotic effect, is the reason for the lack of specificity. At a pressure of 1 atmosphere the action of nitrogen and helium are also similar on newborn and adult rats. These results are unlike those obtained with pressures greater than 1 atmosphere where nitrogen has a specific narcotic effect on the central nervous system (7).

The tolerance is not the same to all the gases studied. With carbon dioxide the survival period of the infant, though shorter than with nitrogen, was nevertheless still much longer than that of the adult in carbon dioxide. Among the factors which may explain this diminished survival time of the infant are the acidity of carbon dioxide and its narcotic action. Cyclopropane similarly shortens survival and exerts profound depression of respiration. The newborn rat in cyclopropane exhibits prolonged periods of apnea.

It is worthy of note that despite respiratory movements by infant rats, in some cases for 40 minutes, when submerged under water no immediate untoward effects are observed. From these experiments it may be seen that at least in the newborn rat respiratory movements in water for a period of 40 minutes have no deleterious action on the continued function of the respiratory mechanisms.

Since the newborn is more tolerant to anoxia than is its mother, it would be surprising if this same phenomenon did not hold antepartum. In our experiments with pregnant rats and cats, which were subjected to 5 per cent oxygen in nitrous oxide, a concentration lethal to the mother in approximately 10 minutes left the fetus capable of spontaneous respiration as observed after delivery by Cesarean section. These infants in many instances were making respiratory movements in utero. An accessory factor making for the survival of the infant during hypoxia may be the character of fetal hemoglobin which takes up relatively large amounts of oxygen even at low pressures of that gas.

The long period of tolerance to nitrogen of infant dog and rat facilitates the observation of respiratory changes in response to anoxia. They disclose a resolution of the phylogenetic development of the respiratory mechanisms. First apneusis develops with a long inspiratory phase indicating a depression of the pneumotaxic centers in the pons and changes of vagal activity (8). Next the apneusis becomes biphasic in character, as the long inspiration is divided in two by a short expiratory effort. Apneusis is succeeded by gasping respiration as only the lower medullary centers are left functioning. Finally after spontaneous respiration ceases there is a period during which respiratory gasps may be evoked by peripheral stimulation. If at any time the animals are permitted to respire air a recapitula-

tion of the respiratory changes is exhibited in the reverse order to that which is produced by anoxia.

In a consideration of the factors which permit the prolonged survival of the infant, the observation that increase of the temperature shortens survival time indicates the importance of metabolic rate. Thus the poikilothermia exhibited by the newborn rat may be considered a protective mechanism. The studies of the cerebral metabolism indicate that the low rate in the newborn may facilitate survival. For example, in the rat after the 10th day postpartum when the rate of cerebral metabolism begins to rise rapidly the survival period approaches the short adult time. Brain metabolic rate is not the only factor for it does not undergo significant changes during the first 9 days of life, a time during which tolerance to anoxia decreases rapidly. Since the puppies in nitrogen survive despite anoxia, it is obvious that there must be anaerobic sources of energy. These anaerobic mechanisms will be discussed in the subsequent paper.

SUMMARY AND CONCLUSIONS

A study is made of the tolerance to anoxia of the adult and infant of various species, rat, dog, cat, rabbit and guinea pig. Anoxia was produced by the respiration of nitrogen, nitrous oxide, helium, carbon dioxide, and cyclopropane and submersion in water. The newborn exhibit an extraordinary tolerance in comparison with the mature animals of the same species. The period of tolerance, however, is not the same in the various species studied being longest in the physiologically immature newborn rat and shortest in the comparatively mature guinea pig. Blood studies reveal that newborn dogs survive despite the fact that their arterial blood contains practically no oxygen after the first few minutes of anoxia. Among the factors permitting survival of the newborn rat and puppy is poikilothermia, a fall of temperature diminishing the metabolic demands. Another factor is the lower cerebral metabolic rate demonstrated in the rat and dog. Fetuses delivered from mothers who had succumbed to anoxia produced by inhalation of 5 per cent oxygen in nitrous oxide or the injection of cyanide respired spontaneously on delivery and some were raised by foster mothers.

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THE EFFECT OF ANOXIA ON THE ABSORPTION OF GLUCOSE AND OF GLYCINE FROM THE SMALL INTESTINE[1]

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Cori (1925) has shown that the absorption of glucose (and galactose) is considerably more rapid than that of other monosaccharides such as mannose and xylose. He concluded that an active process is involved in the absorption of the former. Willbrandt and Laszt (1933) found that monoiodoacetate reduced the rate of absorption of glucose to that of mannose and xylose, indicating that the process involved phosphorylation of the glucose molecule. This work has been amply confirmed (Verzar and McDougall, 1936). Furthermore, Reiser (1940) found that during glucose absorption the inorganic phosphorus content of the intestinal mucosa was decreased, while that of the ester phosphorus was increased.

The Höbers (1936) found that the absorption of various amino acids was faster than their molecular mobility would indicate, when compared with other nitrogenous organic compounds, and concluded that some process other than simple diffusion was active in absorption of amino acids.

The present experiments were undertaken to determine to what extent, if any, these apparently active processes involved in the absorption of glucose and amino acid are affected by oxygen want.

METHODS. Pairs of dogs of as nearly the same weight as possible were used, one dog of a pair as a control and the other subjected to anoxia in a low pressure chamber. Partial pressures of oxygen used were 117, 94, 80, 63 and 53 mm. Hg, corresponding to altitudes of 8,000, 14,000, 18,000, 24,000 and 28,000 feet respectively.

Anesthesia was induced with ether and continued with sodium barbital, 220 mgm./kgm. A loop of intestine was prepared which consisted of the ileum and most of the jejunum. Loops in pairs of dogs were measured to be the same length. When the substance to be absorbed was glucose, the loop was washed with isotonic saline; for glycine absorption, it was washed with isotonic glucose. The intestine was gently stripped between the fingers to remove all washings.

Glucose absorption: Isotonic glucose solution (5.4 per cent) at 38°C.

[1] Aided by a grant of the Ella Sachs Plotz Foundation.

was placed in the loop, in quantity sufficient to fill but not distend it; it was then tied off and returned to the peritoneal cavity. The experimental animal was placed in a low pressure chamber for ninety minutes, the control being kept at atmospheric pressure for the same time. The solution remaining in the intestine was then removed and measured. A sample was diluted and analyzed for glucose by the method of Folin and Wu.

Glycine absorption: The procedure was the same as above, using isotonic glycine (2.3 per cent), which was allowed to remain in the loop for thirty minutes. Analysis for glycine was by Danielson's modification of Folin's method (Danielson, 1933).

At least eight, usually ten or more dogs were used at each barometric pressure for each substance. Nearly all the experimental animals were

TABLE 1

Effect of anoxic anoxia on the absorption of glucose and glycine

	OXYGEN TENSION																
	155 mm. (Control)		117 mm.			94 mm.			80 mm.			63 mm.			53 mm.		
	Approximate altitude																
	800 ft.		*8,000 ft.*			*14,000 ft.*			*18,000 ft.*			*24,000 ft.*			*28,000 ft.*		
	Number of animals	Per cent absorption	Number of animals	Per cent absorption	p*	Number of animals	Per cent absorption	p	Number of animals	Per cent absorption	p	Number of animals	Per cent absorption	p	Number of animals	Per cent absorption	p
Glucose	51	66.2	8	59.3	0.39	9	59.9	0.43	10	64.1	0.77	10	65.1	0.89	25	74.5	0.09
Glucose solution	51	58.2	8	51.4	0.48	9	43.7	0.14	10	53.9	0.62	10	51.9	0.51	25	68.1	0.09
Glycine	68	56.1	10	60.6	0.49	9	55.1	0.88	20	61.6	0.25	20	62.1	0.18	10	39.4	0.010
Glycine solution	68	47.6	10	52.3	0.51	9	45.4	0.78	20	54.8	0.19	20	53.8	0.25	10	30.3	0.019

* Probability of the difference from the control occurring by chance. Should be less than 0.05 to be significant (Fisher, 1932).

paired with controls, which resulted in large control series for both glucose and glycine.

RESULTS. The results obtained are summarized in table 1. It can be seen that both glucose and glycine are absorbed from isotonic solution faster than the water in which they are dissolved. The rate at which glucose is absorbed is depressed slightly at the relatively higher pressures used, practically unchanged at intermediate ones, and increased at the lowest (oxygen tension, 53 mm. Hg, a simulated altitude of 28,000 ft.). None of these changes is statistically significant, although the increase in absorption at 53 mm. Hg oxygen tension is nearly so.

The absorption of fluid roughly parallels the absorption of glucose, except at 94 mm. Hg oxygen tension, where it is depressed, though not significantly, more than glucose absorption.

The rate at which glycine is absorbed is essentially unchanged until an oxygen tension of 53 mm. Hg is reached, where it is significantly depressed. Again the fluid absorption roughly parallels the absorption of the solute, at a somewhat slower rate.

DISCUSSION. Since the increase in absorption of glucose at 53 mm. Hg oxygen tension was quite marked but not statistically significant, an attempt was made to determine whether or not it was genuine.

Originally ten dogs had been subjected to this pressure; more experiments were done; but when a total of twenty-five dogs had been used without any important change in the results, the attempt was abandoned. After the first ten experiments, Fisher's formula showed seven chances in a hundred for the results occurring by chance. When the series of twenty-five were analyzed, the result was nine chances in a hundred. It is not practical to work with barbitalized dogs at any lower oxygen tension, as at 53 mm. Hg about one fourth of the dogs used die before the experiment can be completed. However, there is certainly no decrease in absorption at 53 mm. Hg partial pressure of oxygen.

Colowick, Welch and Cori (1940a) found that in kidney extracts oxidation of a dicarboxylic acid is necessary for phosphorylation of glucose. They further state (1940b) that phosphorylation precedes this oxidation, the latter process being necessary for the continuance of the reaction. They found this to be true also for brain extract.

If absorption of glucose by the intestinal mucosa is dependent on phosphorylation, it is rather surprising, in view of the above related facts, that anoxia does not retard its absorption even in the group of animals subjected to very severe degrees of anoxia. It appears likely, therefore, that phosphorylation in the intact mucosa may depend on a somewhat different mechanism than that investigated by Colowick et al. Gill and Lehman (1939), for instance, report that the formation of the Robison ester from glycogen is inhibited by oxidizing agents and increased by reducing agents.

The marked depression of the absorption of glycine at 53 mm. Hg partial pressure of oxygen suggests the possibility that an oxidative process may be directly involved in the absorption of glycine; certainly it appears that the process is a different one than that involved in the absorption of glucose.

An interesting point is the close parallelism between the absorption of these substances and the water in which they are dissolved. It appears that anything which alters the rate of absorption of the solute, at least when it is present in an isotonic solution, alters in a similar manner the absorption of the solvent. We have noticed this phenomenon in working with substances other than glucose and glycine.

In studying factors affecting absorption, the question always arises as to whether or not changes in the circulation are involved. There seems to be little doubt that cardiac output is increased in anoxic anoxia (Har-

rison et al., 1927; Strughold, 1930), but whether or not there is increased blood flow in the splanchnic area does not seem to have been determined. In the experiments herein reported, since anoxia produced different effects on the absorption of different substances under the same conditions, it is believed that circulatory changes were not an important factor.

SUMMARY

1. Anoxia up to and including 53 mm. Hg partial pressure of oxygen does not alter significantly the absorption of glucose from the small intestine of the dog.

2. Anoxia at 53 mm. Hg oxygen tension, but not higher partial pressures, significantly depresses the absorption of glycine.

3. The experiments suggest that possibly an oxidative process is directly involved in the absorption of glycine. The significance of the lack of effect of anoxia on glucose absorption is discussed.

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THE COLLAPSE FACTOR IN THE MEASUREMENT OF VENOUS PRESSURE

The Flow of Fluid through Collapsible Tubes[1]

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If veins were rigid tubes, then a change in mean right auricular pressure would cause a corresponding change in the peripheral venous pressure, provided the velocity of blood flowing along the veins remained constant. However, veins are not rigid, but collapsible, and it has been shown by Lyon, Kennedy and Burwell (1938), and by Holt (1940), that peripheral venous pressure, referred to the level of the heart as zero, is increased when the vein is above heart level because the veins collapse. Carrier and Rehburg (1923) have also shown that the collapse of peripheral veins and capillaries may maintain capillary pressure at a high level when the capillary is above heart level.

Since veins are collapsible tubes, and the pressure in the right auricle is generally agreed to be subatmospheric, and there is a small positive tissue space pressure around the veins tending to collapse them, it was thought that changes in mean right auricular pressure might not affect peripheral venous pressure because the veins just before entering the chest might be partially collapsed.

Methods and results. When the veins entering the upper end of the thoracic cage were dissected out in the living dog, they were seen to be normally partially collapsed or to dilate and collapse synchronously with respiration. When the animal breathed air which was under a negative pressure the veins were seen to collapse more completely, and when air under a positive pressure was breathed the veins became dilated. The inferior vena cava was seen to collapse, after dissecting the liver away from it, when air under a negative pressure was breathed, but was not seen to collapse with normal respiration.

In ten barbitalized dogs with the chest closed, and placed in the supine position, mean peripheral venous pressure was measured in the femoral,

[1] A preliminary report of this work was given at the meeting of the American Physiological Society in Chicago, 1941.

cephalic, or jugular vein by a modification of the method of Moritz and Tabora (1910). At the same time mean right auricular pressure was measured by means of a saline manometer connected to a cannula that passed into the right auricle by way of the external jugular vein. The venous pressures were referred to the level of the cannula tip in the right auricle as zero. In some cases peripheral venous and auricular pressure fluctuated several millimeters with each respiration; in these cases the pressures were read at the peak of inspiration and at the peak of expiration (fig. 1). The trachea was cannulated and connected to a breathing cham-

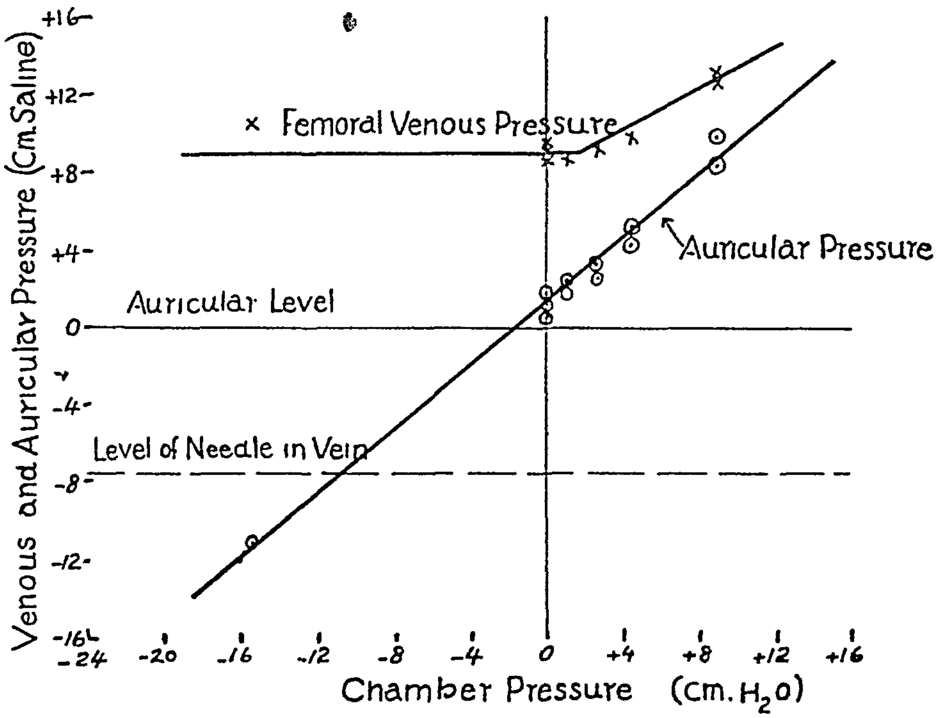

Fig. 1. The effect of changing the breathing chamber pressure on right auricular and femoral venous pressure. +, above atmospheric pressure. —, below atmospheric pressure.

ber in which the pressure was varied between twenty centimeters of water above atmospheric and twenty centimeters below. The chamber was built like a spirometer and had a volume between twelve and fifty liters in different experiments. The spirometer was ventilated with fresh air at a rate of fifteen liters per minute. The dog breathed from the chamber continuously, and when the pressure in the chamber was changed to any positive or negative value five minutes or longer were allowed before the venous and auricular pressure readings were taken. When the chamber pressure was increased the intra-thoracic pressure was increased and auricular pressure rose; when the chamber pressure was decreased the intra-thoracic and auricular pressures decreased.

The results of a typical experiment measuring auricular pressure and femoral venous pressure are shown in figure 1. When the breathing chamber pressure was increased the auricular pressure and peripheral venous pressure increased. When the chamber pressure was decreased the auricular pressure decreased but the peripheral venous pressure did not change. Similar results were obtained on the jugular and cephalic veins with the exception that when the cephalic vein was used a rise in auricular pressure of a few centimeters of saline caused no increase in cephalic venous pressure, but a further rise in auricular pressure caused an increase in cephalic pressure. With the dog in the standing position results similar to those obtained on the femoral vein were obtained on the femoral and cephalic veins with the exception that a slight decrease in auricular pressure generally caused a decrease in peripheral venous pressure, but on further lowering auricular pressure the peripheral venous pressure remained constant. In all of the experiments described the vein was held below heart level. If the vein was held above heart level auricular pressure had to be increased several centimeters of saline before there was any rise in the peripheral venous pressure.

The maintenance of a high peripheral venous pressure when the auricular pressure was low might be explained by an increased rate of flow along the veins resulting from an increased cardiac output when the intra-thoracic pressure was low. In order to rule out this possibility a portion of the venous system of a dead dog was perfused with saline. The brachial, axillary, subclavian and innominate veins on one side, and the superior vena cava were dissected out, left in place, and all side branches entering these veins were tied. This left one large vein, with no open side branches, extending from the antecubital space to the right auricle. The brachial vein was cannulated in the antecubital space and the superior vena cava cannulated at the level of the right auricle. This system was perfused with saline, under a constant head of pressure, through the peripheral end of the brachial vein. The pressure in the peripheral brachial vein and in the superior vena cava was measured. With subatmospheric pressures in the superior vena cava the results were comparable to those obtained in the experiments on the femoral vein in the living dog. A similar experiment was performed on the inferior vena cava with like results. Thus it was shown that it was not necessary for the rate of flow along the veins to increase in order to maintain a constant peripheral venous pressure when the auricular pressure was greatly decreased.

In order to study how the collapse of veins might affect peripheral venous pressure a model (fig. 2) was set up using thin walled rubber tubes to represent veins or using the excised jugular vein of the dog. Water flowed from the Mariot bottle reservoir along heavy walled rubber tubing to a section of collapsible tubing and out through more heavy walled rubber tubing.

The pressures, P_1, above the collapsible segment, and P_2, below the collapsible segment were measured simultaneously with the rate of outflow. The collapsible segment was surrounded by a glass jacket in which the pressure was varied at will. The factors controlling the rate of flow of water through the collapsible tubes were studied by changing one of the above pressures, keeping the other two pressures constant, and measuring the rate of outflow into a graduate cylinder. In this system, when the outlet tube was lowered to a certain point, the pressure at P_2 became subatmospheric; thus P_2 corresponded to the auricular pressure, and P_1 to the peripheral venous pressure in the dog, while the jacket pressure corresponded to the tissue space pressure.

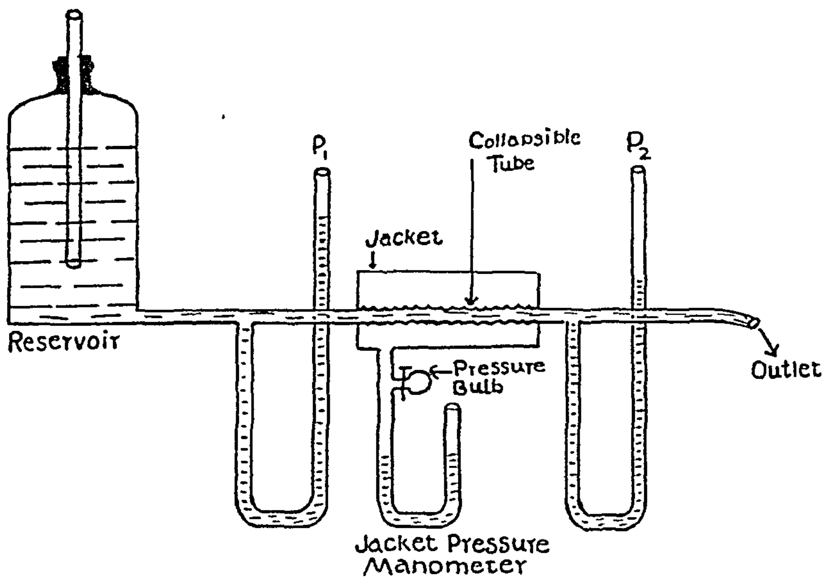

Fig. 2. Model used for studying the flow of water through collapsible tubes

The effect on P_1 of changing P_2 is shown in table 1 A. When P_2 was above jacket pressure (atmospheric in this case) and the tube was dilated, lowering P_2 caused a decrease in P_1, and this continued until P_2 became slightly subatmospheric at which point the tube collapsed. Further decrease of P_2 caused no change or a slight increase in P_1.

The effect of changing P_2 on the rate of outflow is shown in table 1 A. When P_2 was above atmospheric pressure and the collapsible tube was open, lowering P_2 caused an increase in the rate of outflow until P_2 became slightly subatmospheric at which point the collapsible tube started to collapse. As the tube started to collapse it began to pulsate and as P_2 was further lowered the rate of pulsation became more rapid. Finally when P_2 was lowered still further the pulsation apparently stopped and the tube remained partially collapsed. Lowering of P_2, once the tube had begun to

pulsate or to collapse, caused no change in the rate of outflow or a slight decrease.

The effect of changing P_1 on the rate of outflow with the tube open and again with the same tube partially collapsed, is shown in table 1 B. As P_1 increased the rate of flow increased in the open tube and in the partially collapsed tube. In both cases the increase in rate of flow was shown to be a linear function of the increase in P_1 when the data were plotted on graph paper.

TABLE 1

Flow of water through partially collapsed and open tubes

A						B						C		
						Open tube			Partially collapsed tube					
P_1	P_2	P_1-P_2	Flow	Tube	R	P_1	Flow	R	P_1	Flow	R	$J.P.$	Flow	R
cm. H_2O	*cm. H_2O*	*cm. H_2O*	*cc./min.*			*cm. H_2O*	*cc./min.*		*cm. H_2O*	*cc./min.*		*cm. H_2O*	*cc./min.*	
23.7	23.4	0.3	31.5	Open	0.0095	7.3	74	0.012	19.45*	63	0.76	22.3*	30	1.66
14.3	13.9	0.4	36.0	Open	0.011	8.0	182	0.0088	21.0	146	0.34	21.5	75	0.66
0.7	0.2	0.5	45.0	Open	0.011	8.85	276	0.0089	21.6	200	0.25	21.0	98	0.508
0.0	−2.4	2.4	45.5	Pulsating	0.052	9.50	372	0.0083	22.8	279	0.18	20.4	122	0.408
0.3	−12.1	12.4	45.5	Pulsating faster	0.27	10.80	480	0.009	24.0	360	0.14	19.9	175	0.284
1.0	−45.4	46.4	44.4	Collapsed, no pulse	1.04									
1.6	−105.4	107.0	44.0	Collapsed, no pulse	2.43									

A, effect on P_1 and on the rate of flow through the jugular vein of changing P_2. B, effect of changing P_1 on the rate of flow through a thin walled collapsible rubber tube 7 mm. in diameter (in the open tube $P_2 = 6.4$ cm. of water, jacket pressure = atmospheric; in the partially collapsed tube $P_2 = -28.5$ cm. of water, jacket pressure = 19.9 cm. of water). C, effect of changing the jacket pressure on the rate of flow through the partially collapsed rubber tube ($P_1 = 21.3$ cm. of water, $P_2 = -28.5$ cm. of water). *J.P.*, jacket pressure. *R* resistance. −, minus.

* It took approximately 1 cm. water pressure to overcome the elasticity of the rubber tube and to collapse it, thus the jacket pressure is slightly higher than P_1 here.

The effect of changing the jacket pressure on the rate of flow when P_1 and P_2 were kept constant is shown in table 1 C. As the jacket pressure decreased the flow increased.

The fact that the rate of flow did not increase when P_2 was decreased, once the tube was partially collapsed (table 1 A), means that the resistance to flow through the partially collapsed tube increased as P_2 decreased. The resistance to flow may be calculated from the data in table 1 using the conventional formulation, $R = \frac{P_1 - P_2}{\text{Flow}}$. With the viscosity constant, as is the case in these experiments, the increase in R as the tube collapses must be caused by a decrease in the cross-sectional area of the collapsed segment, to an increase in turbulence, to an increase in the length of the

partially collapsed segment, or to a combination of these factors. No increase in the length of the partially collapsed segment was detected.

Table 1 A shows that in the partially collapsed tube the resistance increased as P_2 decreased, and table 1 B that the resistance decreased as the flow increased and as P_1 increased, and table 1 C that the resistance decreased as the jacket pressure decreased.

The length of the collapsible tube had little effect on the flow, since similar results were obtained on a collapsible tube 80 cm. long and on another 2 cm. long. When the longer tube collapsed the collapsed segment was always at the downstream 1 or 2 cm. of the tube, the rest of the tube remained open unless the flow through the system was very small. It appears that it was only necessary for the collapsible tube to be long enough and relaxed enough to collapse to give the effects of collapsible tubes described above.

The pressure: flow graphs that were plotted from the data in table 1 B were straight lines, whereas in experiments on smaller tubes, such as the jugular vein, the pressure:flow graphs were smooth curves convex toward the flow axis. This apparently is the result of the abrupt change in cross-section, which occurs at the point where the cannula tips are tied into the vein, causing the flow to be turbulent (Dodge and Thompson, 1937). However, qualitative results similar to those described in table 1 were obtained on these tubes.

Discussion. The fact that peripheral venous pressure remained constant when auricular pressure was decreased greatly and in some cases when auricular pressure was increased a small amount may have been caused in part by the change in auricular pressure being associated with a change in cardiac output and with a change in the rate of flow of blood along the veins. However, since the veins were seen to collapse, and since similar results were obtained on the excised jugular vein in a model, and on the dead dog's venous system acting as a model, it would appear that the collapse of the veins near the heart was an important factor in maintaining the peripheral venous pressure normal when auricular pressure changed.

Since auricular pressure may change independently of a change in peripheral venous pressure, it appears that the usual clinical measurement of venous pressure may give little indication of the pressure in the right auricle.

In the collapsible tubes studied here, the resistance to the flow of water decreased as the head of pressure, P_1, increased and as the jacket pressure decreased. Lowering the pressure on the downstream side of the collapsible tube increased the resistance to flow. Thus collapsible tubes differ from rigid tubes in that the resistance to flow remains constant in rigid tubes as the pressure-drop across the tube changes (so long as the flow is not

turbulent), while in collapsible tubes the resistance changes as the pressure-drop across the tube changes. Although the increased resistance to flow offered by the partially collapsed tube might be caused in part by an increase in turbulence at the partially collapsed segment, it seems certain that part of the increased resistance is caused by a decrease in cross-section of the segment, since the cross-section is observed to decrease in size when the tube collapses and appears to decrease still further the more the tube becomes collapsed.

It should be noted that in the graphs plotted from the data in table 1 B the pressure:flow line was steeper in the collapsed tube than in the open tube, that is, it took a greater increase of P_1 in the partially collapsed tube than in the open tube to cause a given increase in flow. The pressure: flow lines became steeper as the collapsible tube became more collapsed, i.e., at higher jacket pressures. This was the case in both the jugular vein and the thin walled rubber tubes. The reason for the greater slope of the pressure:flow line when the jacket pressure is higher is not clear.

Since the collapsible tubes studied show pulsation in the early part of the collapsing process, there is the possibility that some part of the venous pulse seen in the veins entering the chest may be caused by this type of pulsation. Also, since the veins entering the chest do collapse when there are moderate negative pressures in the right auricle, and since it has been shown that decreasing the pressure on the downstream side of a partially collapsed tube does not increase the rate of flow through the tube, it seems that this collapse of the veins entering the chest may be a mechanism which insures a normal flow of blood into the heart but prevents over-filling of the heart and intra-thoracic vessels when large negative pressures are present in the chest as in Müller's experiment or when a deep inspiration is taken.

SUMMARY

Right auricular and peripheral venous pressures were measured in dogs breathing from a chamber in which the pressure varied between 20 cm. of water above atmospheric and 20 cm. below. It was shown that when auricular pressure was decreased greatly and in some cases when auricular pressure was increased slightly the peripheral venous pressure remained constant. In most cases when auricular pressure increased the peripheral venous pressure was increased.

The flow of water through collapsible tubes such as the jugular vein of the dog was studied in a model. When fluid is flowing through a partially collapsed tube, increasing the pressure on the upstream side of the partially collapsed segment decreases the resistance to flow through the collapsible segment and increases the rate of flow, whereas lowering the pressure on the downstream side of the collapsible segment increases the resistance to flow through the collapsible segment and either does not change the rate of

flow or decreases it slightly. An increase in the jacket pressure around the collapsible tube increases the resistance to flow through the collapsible segment and decreases the rate of flow.

As a collapsible tube, having fluid flowing through it, starts to collapse it pulsates and as it becomes more collapsed the pulsation increases in rate on further collapse the pulsation apparently disappears.

The length of the collapsible tube is not important in controlling the length of the partially collapsed segment. It appears to be only necessary that the tube be long enough and relaxed enough to collapse in order to give the results described, and any length of collapsible tube greater than this length merely acts as a dilated or rigid tube.

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EFFECT OF GELATIN UPON MUSCULAR WORK IN MAN

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Ray et al. (1939) claimed that gelatin feeding caused an increase of 37 to 240 per cent in the amount of work performed by male subjects. Since their experiment lacked controls, and the effect of training was underestimated, it seemed of interest to undertake further experimentation with gelatin feeding.

Five series of tests were performed. *Controlled diet:* 1, county jail inmates, bicycle riding; 2, campers, swimming. *Non-controlled diet:* 3, YMCA members, heavy-weight lifting; 4, YMCA members, wall-weight pulling; 5, college students, bicycle riding.

The reasons for using groups with non-controlled diet were: 1, it was desirable to repeat the experiment in a manner approximating the procedure of Ray and his co-workers, and 2, it was impossible to control the diet of some men beyond a request that they remain on their usual diet.

Experiments with county jail inmates. Twelve men, free from disease, were selected from a large number of volunteers. Eleven of the men were white and one was colored. The ages varied from 18 to 50 years.

The whole experiment may be subdivided into three periods:

1. Four weeks (one hour a day, five days a week) of building-up exercises for the legs and back muscles, because some of the men were not fit to work on the ergometers due to long enforced inactivity.

2. Five weeks of preparatory work on ergometers, with a gradual increase in rate of work starting from 0.06 H.P. The men worked five days a week (Saturdays and Sundays were excluded).

3. Period of actual experimentation, which varied from six to twelve weeks. The rate of work varied for different men from 0.159 to 0.261 H.P. (table 1). Work started after breakfast and continued as long as the subject could maintain a predetermined number of pedal revolutions per minute. When the riding continued for several hours, a subject might dismount and spend half a minute in going to the toilet (located in the experimentation room), but this did not happen for every subject every day.

Bicycle ergometers were used. Resistance was supplied by an automo-

[1] Now with the Scientific Bureau, Bausch & Lomb Optical Co., Rochester, N. Y.

bile brake lining around the fly-wheel. The upper end of the brake belt was attached to a spring scale with one ounce divisions, and weights were attached to its lower end. For an easier control of resistance which fluctuated occasionally, a simple device shaped like a trident was suspended by the middle prong from the brake cord. At least one pound in ounce weights was placed on its outer prongs; when the brake resistance fluctuated, some weights were either removed or added as the case might be. Hexagonal nuts each weighing one ounce were found to be convenient for this purpose.

TABLE 1

The essential data for the prison inmates working on the bicycle ergometers

The number of pedal revolutions per minute was 70 in all cases except that of no. 7, for whom it was 60 r.p.m. A rather high initial riding time and work are due to five weeks of preparatory training on the ergometers; during the fifth week the same rate of work was used as indicated on the table, but during this period the men rode from two to three times daily without reaching a point of exhaustion.

SUBJECT NO.	AGE	HEIGHT	WEIGHT	RATE OF WORK		WORK DONE IN FT.-LBS. PER DAY		RIDING TIME		PER CENT OF IMPROVEMENT
				Ft.-lbs. per min.	Horsepower	Initial	Maximal	Initial	Maximal	
			lbs.					*hrs. min.*	*hrs. min.*	
1	33	5′ 9″	170	6000	0.182	180,000	2,160,000	0 30	6 00	1100
2	41	5′ 6″	136	6000	0.182	240,000	576,000	0 40	1 36	140
3	44	5′ 7″	144	6000	0.182	72,000	1,896,000	0 12	5 16	2533
4	20	6′ 0″	178	7150	0.217	143,000	1,787,500	0 20	4 10	1150
5	28	5′ 2″	134	6000	0.182	60,000	1,290,000	0 26	3 35	727
6	24	5′ 11″	160	7000	0.212	140,000	1,680,000	0 20	4 00	1100
7	18	5′ 8″	121	5600	0.170	156,800	196,000	0 20	0 35	75
8	22	5′ 8″	174	6000	0.182	132,000	750,000	0 22	2 05	468
9	50	5′ 11″	184	5250	0.159	168,000	840,000	0 32	2 40	400
10	27	5′ 6″	140	7500	0.227	37,500	1,675,500	0 05	3 46	4420
11	23	5′ 11″	148	7150	0.217	228,800	2,659,800	0 32	6 12	1062
12	33	5′ 7″	204	8600	0.261	817,000	1,548,000	1 35	3 00	89

Nine ergometers were used simultaneously. Each was equipped with a speedometer and distance meter. A metronome was also used to double-check the rate of pedaling.

Diet. All inmates were on the same basic diet, although the amount of bread consumed varied somewhat, since they could have all the bread they wanted. Subject 2 received a small additional quantity of milk daily to provide more liquid in his diet because of dental work in progress.

Work on jail inmates started with body-building exercises which continued for four weeks and comprised the first period of the experiment. After this, the preparatory work on ergometers began, all subjects starting

without gelatin. Three and a half weeks later 6 of the men were given 32 grams of gelatin in grapefruit juice, and 6 others were given an equal amount of a cereal (farina) in the same way. The latter was called "concentrated gelatin" to explain the insolubility of farina in the grapefruit juice, and to assure these men that they were merely getting another kind of gelatin.

The result of this change in diet was immediate. Every subject showed an improvement within an hour after ingestion of either gelatin or cereal, indicating that the effect was purely psychological.

The performance continued to increase so rapidly that it became necessary to increase the loads to the values shown in table 1 in order to keep the riding time within reasonable limits. We also discontinued gelatin feeding and put every man on farina. This part of the experiment *perforce* had to be regarded as a part of the preparatory work, constituting the second period.

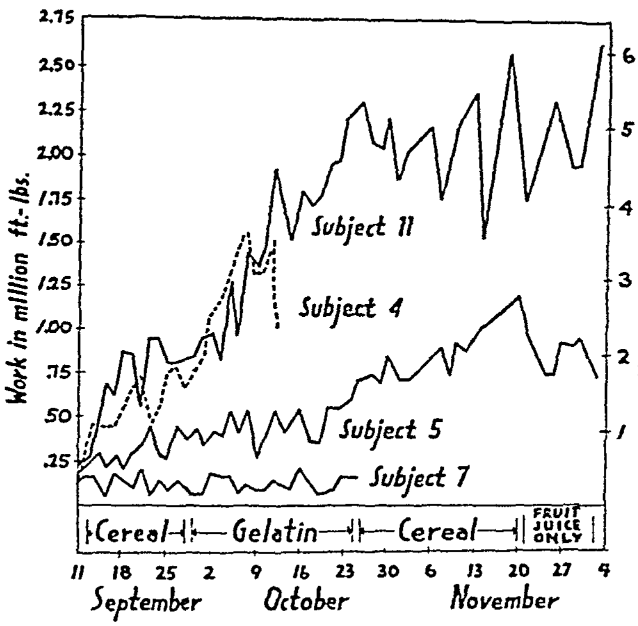
One week was allowed the men to become familiar with the new heavier loads; after this we started the third, final period of the experiment. All men worked on farina diet from four to seven weeks, then they were given gelatin. This diet was continued for some time and then gelatin was again withdrawn, and in its place farina, fruit juice or neither was given. In no case did the men know when they were getting gelatin or a substitute.

Results. From table 1 it may be seen that increase in performance varied from 75 to 4420 per cent. The latter figure may appear to be large, but as a matter of fact it should have been larger. During the preliminary training on the ergometer most of the men could not last more than ten minutes with lighter loads. In order to check the difficulties of the ergometer work we tried it ourselves. It took us two weeks to pass the five minute mark at 0.182 H.P.

Figure 1 presents four typical curves of performance. Subject 11 seemed to improve markedly with the addition of gelatin to his diet. Yet it would be unwarranted to conclude that this was a gelatin effect, because subject 4, who had no gelatin, showed an identical gain at the same time; and subjects 5 and 7 were not affected although gelatin was given to them. A sudden drop in the performance of the subject 4 was a result of a knee injury. The performance of the subject 5 continued to improve after the withdrawal of gelatin and the substitution of cereal. But when cereal, known to him as "concentrated gelatin" was withdrawn, performance immediately dropped, and the subject complained that he was getting too tired without the "gelatin."

It is impossible to note any special effect of gelatin upon the work output. Addition of gelatin to the diet did not improve performance, nor did the withdrawal of gelatin make it worse. All increase in performance was undoubtedly a result of training.

Validity of the test. While work of long duration may be cut short by psychological factors, as suggested by Simonson and Sirkina (1933), special efforts were made to elicit maximal performances. The prisoners preferred exercise at an hourly rate of pay to idleness in their cells. A competitive spirit was aroused by having them work side-by-side. At the end there was subjective and objective evidence of exhaustion. One educated and intelligent subject remarked that he kept on until he became dizzy. At the end of a five-hour ride he was unable to talk and his reactions were



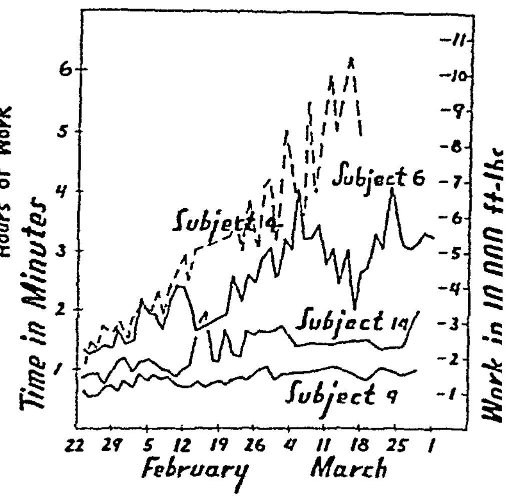


Fig. 1. Typical curves of prolonged work on bicycle ergometers. Subjects—jail inmates. Subjects 4 and 11—0.217 H.P.; subject 5—0.182 H.P. and subject 7—0.170 H.P. Subjects 5, 7 and 11 received gelatin as indicated on the graph. Subject 4, whose curve follows that of no. 11, received no gelatin. The drop in his curve was due to a knee injury.

Fig. 2. Typical curves of short intensive work on bicycle ergometers. Rate of work—0.506 H.P. Pedal revolutions—117 per minute. Subject 4, whose performance is the best, received no gelatin. A drop in performance in curves 6, 14 and 9, which occurred between March 4 and 18, was the effect of college term examinations.

slow. It was noted that after a rest in a reclining position some subjects had to lift their legs by hand as they arose from the bed. These observations indicate to us that physiological rather than psychological factors were responsible for the steady increases in performance.

Metabolism studies. The metabolism of five men was studied after ten weeks of training. Two of the men were on gelatin at that time and three were not. Metabolism was tested: 1, lying; 2, sitting on bicycle; 3, pedaling bicycle without load (free wheeling); 4, pedaling bicycle with full load; and 5, recovery after exercise. During the work with full load, expired air was collected for 4 to 5 minutes each half-hour. The Douglas-Haldane

method was used. No relation between metabolism data (table 2) and gelatin feeding was noticed.

Source of energy. The appetite of all men increased with the increase in riding time. Since bread was supplied in liberal amounts at each meal, the subjects ate it in large quantities. When, for instance, subject 11 passed five hours of riding, he would take 12 to 14 slices of bread with his breakfast, 14 to 19 slices of bread with lunch and a pound loaf with his supper; he was eating from one to two pounds more of bread than before. Assuming that a pound of bread supplies 1180 calories, an extra supply of 1180 to 2360 calories was provided. The amount of net energy spent on the bicycle by subject 11 in five hours was 1716 calories; thus it is obvious that the principal source of *extra* energy for the prolonged riding was the

TABLE 2

Summary of data obtained in work metabolism tests

Subjects were jail inmates working on bicycle ergometers. In calculating the net amount of energy used and efficiency, free wheeling was taken for the base.

SUBJECT NUMBER AND DIET	O_2 CONSUMPTION PER MIN. DURING WORK	OXYGEN DEBT	RESP. QUOTIENT	WORK PER MINUTE	NET ENERGY USED PER 1000 FT.-LBS. OF WORK	NET ENERGY USED PER MINUTE	EFFICIENCY PER CENT OVER FREE-WHEELING
	liters	*liters*		*ft.-lbs.*	*Calories*	*Calories*	
1. Gelatin	1.9	2.32	0.96–0.98	6000	1.13	6.78	29
3. Gelatin	1.7	0.224	0.91–0.97	6000	1.05	6.30	31
4. No gelatin	2.3	2.70	0.91–0.98	7150	1.08	7.72	30
5. No gelatin	1.7	0.200	0.92–0.99	6000	1.07	6.42	30
11. No gelatin	1.4	2.00	0.97	7150	0.80	5.72	41

extra amount of bread consumed. This was also substantiated by his respiratory quotient of 0.97 during work.

Efficiency of work. For calculation of energy used for overcoming the brake resistance we accepted the metabolism at free-wheeling as the point of reference. With one exception, the efficiency of the men was about the same for all, varying from 29 to 31 per cent. The exception was subject 11 whose efficiency was 41.0 per cent. These figures lie within the range found by Benedict and Cathcart (1913). On our subjects it was impossible to notice any effect of gelatin feeding upon the efficiency. The high efficiency of subject 11 should be attributed to "individual difference."

Immediate effect of free wheeling upon work with full load. During the first two or three metabolic tests, we encountered a peculiar psychological effect which occasionally compelled us to discontinue the testing for that day. After 15 to 20 minutes of free wheeling, the subjects found it extremely hard to work with their ordinary load. Some of the men gave

up in 10 minutes, insisting that the weight had been doubled. They exhibited all the symptoms of extreme fatigue. After checking the weights and proving that they were the same as always, and after allowing a 10 to 15 minute rest, the men were able to resume their work; but on such a day they could never equal their expected riding time. In further tests we found that by a preliminary explanation and by encouragement it was possible to eliminate this factor and to make the men work as usual. Even then there was a certain critical point which seemed difficult to pass; however, after it had been passed, work seemed to be easy. This period varied from 10 to 30 minutes after beginning of the riding, being rather constant for each man. For instance, subject 1 had his critical period 25 to 30 minutes after the start.

College students. The subjects for this series of experiments were 16 Springfield College students, ranging in age from 18 to 26 years, and in weight from 139 to 184 pounds. The exercise consisted of riding on bicycle ergometers at a rate of 16,700 ft-lbs. per minute, or 0.506 H.P., and 117 pedal revolutions per minute. They worked five days per week, riding twice a day with a five-minute rest between the rides. After six weeks of experimenting it was observed that improvement in performance in most men was small. Upon the suggestion of D. B. Dill it was decided to introduce a change in testing procedure.

Four men, nos. 4, 6, 12 and 13, continued as before, but the other twelve men worked at the original rate of 0.506 H.P. only twice a week, and on the other three days they rode only once a day at a rate of 0.329–0.354 H.P.

Rate of work. Although the rate of 0.506 H.P. of work is quite large, some of the men, nevertheless, could keep up this rate for several minutes; the maximum was 6 minutes 18 seconds.

From the article by Ray et al. (1939) it is impossible to see the real work output which was used in their study. Evidently their ergometer had a poor efficiency, since an output of 60 watts (0.08 H.P.) was able to fatigue the men in a short time.

The rate of work of women subjects in the experiments of Hellebrandt, Rork and Brogden (1940) approximates that found in our investigation. With allowance for sex, an output of 220 to 290 watts (0.29 to 0.39 H.P.) is a large output for a woman.

Diet. The basic diet of the men could not be controlled outside of a request that they eat about the same during the course of the experiment. For control purposes, they were divided into several groups (see table 3).

Results. Examination of table 3 will show that the per cent of improvement in work varied from 49 to 334. The man who improved most received no gelatin at all. Unfortunately he had to quit the experiment at the end of the eighth week due to an urgent call from home.

We should like to call attention at this time to a possible error that may

TABLE 3

Time and per cent of improvement over the daily average work during the first week in college students

Subjects working on bicycle ergometers at 0.506 H.P. and 117 pedal revolutions per minute. The duration of the experiment was 10 weeks.

GROUP	SUBJECT NO.	RIDING PERFORMANCE		IMPROVEMENT
		Ave. 1st week	Maximum attained	
		seconds	*seconds*	*per cent*
1 Gelatin throughout experiment	13	56	240	330
	14	52	122	135
	15	37	66	78
	16	38	77	103
Average		45.75	126.2	161.5
2 Farina 4 weeks; gelatin 6 weeks	1	53	95	79
	2	42	73	74
	3	46	70	49
Average		47	79.33	67.33
3 Blank 6 weeks; gelatin 4 weeks	5	26	63	142
	12	56	185	230
Average		41	124	186
4 Farina throughout experiment (no gelatin)	6	80	246	208
	7	41	72	76
	10	37	88	138
	11	42	83	98
Average		50	122.25	130
5* Farina 4 weeks; blank 4 weeks (no gelatin)	4	87	378	334
6 No gelatin; no farina	8	61	128	110
	9	37	63	70
Average		49	95.5	90

* Had to discontinue at end of eighth week for reasons not involving health.

occur in the interpretation of results obtained in experiments similar to ours if composite curves representing the average performance of the different groups are used. If by chance we used only twelve men, and groups 2 and 4 (see table 3) were absent, then this table could have been used as proof of a beneficial effect of gelatin, since the groups 1 and 3 give a higher per cent of improvement than the non-gelatin groups 4 and 6. Only by a comparison of the differences within each group does it become evident that the variation within a group is greater than any variation between groups.

The only conclusion that can be made is that gelatin had no effect upon the work capacity of the men in this experiment. This conclusion is in perfect agreement with the observation by Hellebrandt et al. (1940). Maison (1940) also found no effect of gelatin feeding in four subjects who exercised on a finger ergograph. On theoretical grounds his results may be disputed because of the smallness of the muscle group used. Even if glycine were beneficial, it could have been supplied to the finger muscles at the expense of the other groups. This is why we preferred exercise involving large groups of muscles. On the bicycles not only are the legs involved, but also the trunk muscles, and even the arm muscles are constantly being used.

Daily work done by the College Students is presented in the figure 2. The rate of work was 0.506 H.P. for all men, yet the rapidity and the degree of the improvement varied considerably. This was due to a great extent to a difference in muscular strength. Subjects 4 and 6 were among the strongest men in the group.

In the three lower curves (fig. 2) a depression may be observed between March 4 and 18. This coincided with the period of term examinations held between March 11 and 18. Of sixteen men only three were not affected by examinations.

After completion of a ten-week period subject 6, who belonged to a group not receiving gelatin, continued to work on the ergometer for two more months. At the end of this extra period, his riding time had reached 7 minutes, 30 seconds, which means a work output of 125,310 ft-lbs., an improvement of 463 per cent. His curve showed a continuous rise, but the experiment had to be discontinued because of college final examinations. However, this clearly indicates that the peak in training on the bicycle may not be reached even after 19 weeks of training.

It is interesting to compare these figures with those obtained by Henderson and Haggard (1925) on Olympic oarsmen. They estimated the rate of work done in breaking the world's record for a mile and a quarter in 5 minutes 51 seconds, as being equal to 0.57 H.P. By coincidence, subject 6 was a candidate for the Olympic contests in canoeing.

Performance during the second ride with 0.506 H.P. rate. No effect of gelatin could be observed on recovery from the first ride.

Lighter loads. Three days a week of riding with lighter loads (0.329–0.354 H.P.) did not affect the curve of performance obtained in riding two days a week at a rate of 0.506 H.P.

Experiments with summer camp boys. Thirty boys, ranging from 15 to 17 years in age, were selected in two summer camps. In each camp they were divided into two groups, one receiving 32 to 48 grams of gelatin daily, and the other receiving a non-gelatinous substitute. The rest of their diet was essentially the same for the boys in each camp. They swam daily (60 to 100 yds.) trying to better their time.

The experiments continued for 6 to 8 weeks. No difference was observed between the gelatin and non-gelatin group.

Experiments with heavy-weight lifters. Twelve heavy-weight lifters who were members of two YMCA organizations volunteered to take part in this experiment. They exercised two or three times a week for 10 weeks trying each time to beat their previous records in the nine standard types of weight lifting. The basic diet could not be controlled beyond a request that they use "approximately the same diet" throughout the experiments. The men were divided into two groups: gelatin (32 to 64 grams daily) and non-gelatinous substitute. No difference could be observed between the performance of these groups.

Experiments with wall-weight pullers. Exercises for the arms and shoulder retractors were used, with the height of elevation of the weights kept constant and the rate controlled by metronome. Six men (gelatin 32–64 grams daily, and no gelatin) exercised until they could not maintain the rate. This experiment proved unreliable due to erratic performances. However, no difference was noted in the performance of the two groups.

SUMMARY AND CONCLUSIONS

The effect of gelatin feeding upon muscular performance was tested on five groups of subjects, numbering 76 in all. Exercises used were: work on bicycle ergometers, swimming, weight-lifting and wall-weight pulling. Diet was either fully or partially controlled. Sham gelatin feeding by use of a non-gelatinous substitute was practiced with each group.

1. Twelve county jail inmates, 18 to 50 years of age, exercised on ergometers for from 17 to 22 weeks, 5 times a week. The rate of work varied from 0.159 to 0.261 H.P. Performance improvement ranged from 75 to 4420 per cent; the maximum riding time of one subject reached 6 hours 12 minutes, and the work done on that day was 2,659,800 ft-lbs. Diet was fully controlled. No effect of gelatin feeding could be observed.

2. Sixteen college students between 18 and 26 years of age exercised on bicycle ergometers five times a week for 10 weeks. Their rate of work was

0.506 H.P. Improvement in ten weeks was between 49 and 334 per cent, and in the case of one man who exercised for 9 extra weeks at 0.506 H.P. it reached 463 per cent; the maximum working time was 7 minutes 30 seconds. Diet was not controlled. No effect of gelatin feeding was observed.

3. Thirty campers, ranging from 15 to 17 years of age, swam 60 to 100 yards each day from 6 to 8 weeks. Diet was controlled. No effect of gelatin feeding could be observed.

4. Twelve weight lifters tried to break their previous records in nine standard positions. Diet was not controlled. No effect of gelatin could be observed in 10 weeks.

5. Six men exercised by pulling wall weights 3 days a week, attempting each time to increase the number of pulls. Diet was not controlled. No effect of gelatin could be observed.

6. At the same rate of work, the stronger men improved more than the weaker ones.

7. Metabolism of 5 jail inmates was studied. Efficiency of work varied from 29 per cent to 41 per cent. The maximum amount of net energy spent on daily exercise reached 2441 Calories. The *extra* energy for this work was supplied by the extra amount of bread consumed. No effect of gelatin could be observed in the metabolic tests.

8. College term examinations caused a drop in performance of most of the subjects.

9. A psychological effect of gelatin and sham feeding was observed in jail inmates. The first day they received either gelatin or a non-gelatinous substitute, their performance noticeably increased before either of the substances could have been digested and assimilated.

We wish to thank Mr. Harold LeMaistre, of Sydney University, Australia, for help with metabolic tests of jail inmates.

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ACUTE EFFECTS OF SPINAL CORD SECTION UPON THE PLASMA VOLUME AND BLOOD PRESSURE OF CATS UNDER ETHER ANESTHESIA

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It is well known that surgical or other interruption of spinal pathways is commonly followed by a fall in arterial pressure. The possibility of a change in blood volume occurring under these circumstances has received relatively little attention. Eppinger and Schürmeyer (2) reported a single experiment in which high spinal transection apparently reduced the blood volume of a dog from 1500 cc. to 907 cc. They considered this result as comparable to their findings in animals suffering from various forms of circulatory collapse. Nowak (10), using the congo red dye method, estimated the blood volume of cats before and during spinal anesthesia, and found an average increase of 3 cc. per kgm. body weight. His animals had undergone previous laminectomies and were anesthetized with barbiturates during the spinal block. Goldfarb, Provisor and Koster (3) concluded from data obtained by the brilliant vital red method on surgical patients that no significant blood volume change occurs during spinal anesthesia, although their values for percentage change in individual cases range from −11.9 to +15.7. The three papers just cited deal only incidentally with blood volume or plasma volume changes, and in none of them are the experimental conditions and methods described sufficiently to permit an evaluation of the results.

A correct interpretation of plasma alterations in animals undergoing acute interruption of cord pathways requires that account be taken not only of local modifications within the cord itself, but also of a variety of changes incidental to the experimental procedure. These include premedication, anesthesia, incidental operative trauma, respiratory disturbances, modified sympathetic activity, and a number of other factors. In chronic animals, the effects of muscular disuse and of change in dietary or other habits may be important. Moreover, animals surviving transection or total destruction of the spinal cord even for short periods may regain not only their pre-operative blood pressure levels, but also a limited reflex control of the circulation (6, 13). Accordingly, the writer decided

to use acute preparations, and, as will be seen, to eliminate certain of the disturbing factors. It was hoped that in this way any plasma volume changes which might occur could be referred with some confidence to the effects of cord transection as such. It must be emphasized, however, that the factor of anesthesia has not been eliminated in these experiments, and that the results are therefore applicable only to cats under the conditions described. Obviously the attempt to obtain control values and to transect

Fig. 1

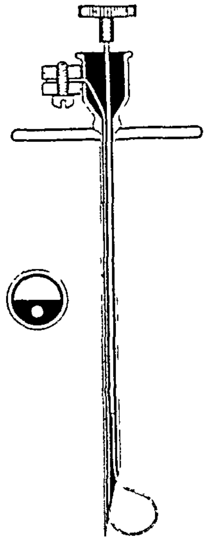

Fig. 2

Fig. 1. Portion of spinal cord of cat 17, showing lesion. Above: dorsal view. Below: ventral view.

Fig. 2. Instrument for destroying portion of cervical cord. Inset on left: cross section through shaft of needle, showing plunger and long arm of wire loop. For further description, see text, p. 4.

the cords of unanesthetized animals could only lead to sympathetic activity far more disturbing than that occasioned by ether.

Methods. The traumatic effects of laminectomy with attendant blood loss and tissue exposure were avoided by the use of a blind puncture instrument (fig. 2). The main part of this device was a four inch, thirteen gauge hypodermic needle. A J-shaped loop of no. 4 music wire was arranged within the shaft of the needle so that it could be anchored by its long arm outside the needle's head, while the short arm engaged a plunger the lowering of which caused the wire loop to emerge from the orifice of the needle and to spread to a diameter of 6 to 8 mm. With the animal under

ether anesthesia, this loop was spread within the substance of the spinal cord, and then rotated by a turning of the needle so as to reduce the cord to a pulp at the level of the puncture. Following the withdrawal of the loop by traction upon its long arm, a heated rod was plunged into the pulped cord through the same needle, in order to coagulate any fibers remaining intact, and, by cauterization, to reduce the danger of infection or of extensive internal bleeding. When the operation is performed at or above the level of the eighth cervical segment, only a slight nick in the skin is necessary for the introduction of the needle. The animal is placed upon its side, with the neck slightly flexed. The neural spine of T_1 serves as a guide for the needle point as far as the ligamentum flavum. When this is reached, a quick thrust will generally send the needle point directly through the center of the cord. Thereupon the loop is spread, rotated, and withdrawn; then cauterization is applied as described above. The entire operation consumes less than two minutes. When a chronic preparation is desired, the method offers the advantage that the period of anesthesia may be kept exceedingly short. Adequate antisepsis may be attained merely by boiling the puncture instrument. Of six chronic spinal cats so prepared, none showed any sign of infection either in the cord or in the superficial tissues. Ten days to several months after the transection, the animals were killed with ether.

Disadvantages of this method include the following: *a*, the destruction of the cord is not invariably complete, and must be checked at autopsy; *b*, the lesion is never sharp and clean-cut, but contused and likely to be somewhat asymmetrical with reference to the long axis of the cord; *c*, the cord may be attacked only where it is not protected by overlapping bony arches—practically, in cats and dogs, the cervical region.

At frequent intervals during each of the experiments, the animal was examined for functional evidence of nervous transmission across the lesion. At no time did such evidence appear. After recovery from ether each cat showed complete volitional paralysis and anesthesia in the trunk and hind quarters. Breathing was entirely diaphragmatic. The nictitating membranes were widely extended. At the conclusion of each experiment, a portion of the cord including the lesion was removed, freed from its outer membranes, and examined grossly. When there was any reason to suspect that complete functional destruction had not been attained, the experiment was discarded. Figure 1 is a photograph of a cord (no. 17) showing a typical lesion. The specimen had been kept in 10 per cent formalin for several weeks.

Plasma volume determinations were made by the use of the dye T-1824, optical densities being estimated spectrophotometrically (4). A control sample of 1 cc. was taken from the right saphenous vein soon after anesthesia had been induced. Following the dye injection, and the cannulation

of the right femoral artery, eight 1 cc. samples were generally drawn from the left saphenous vein. Four of these samples were taken before the spinal transection, and four after, at intervals of about fifteen minutes. The first post-operative sample was usually obtained within five to ten minutes after the transection, the time chosen being that at which the blood pressure appeared to have reached its minimum value. Deviations from a control curve established by the first four dye samples gave the plasma volume fluctuations here interpreted as the result of the spinal section. These deviations were checked by comparison with serum protein and hematocrit determinations. Since the first post-operative sample commonly shows the maximum deviation from the control curve, the experimental change (see table 1) was reckoned from this value. The deviation of the last sample was taken as an index to the extent of return of the plasma volume to its control level.

Proteins were estimated refractometrically in the sera which had previously been used for the dye determinations, the calculations being based upon the factors given for dog serum by Neuhausen and Rioch (9). These data were used only for purposes of comparison with the dye curves, and therefore relative values only were required. Nevertheless, it was thought desirable to check the refractometric estimates against those made by some other method. Through the kindness of Dr. A. Graff, a comparison was made of the values obtained by the use of the refractometer with the results of microkjeldahl analyses, assuming a non-protein nitrogen content of 30 mgm. per 100 cc. The average serum protein concentration in five refractometer determinations was 6.85 per cent. Microkjeldahl analyses of the same sera gave an average protein concentration of 6.81 per cent. The greatest difference between the values given by the two methods for any one sample was 0.06 per cent. It can be shown that a change of 100 per cent in non-protein nitrogen affects the serum protein estimate by only 0.3 per cent. The refractometer estimates, therefore, are probably good approximations of the absolute values. Two hematocrit samples were taken for further comparison with the dye curve. One was included with the last pre-operative, and one with the first post-operative dye sample. The total amount of blood lost in sampling was about 10 cc.

The cats were starved for 18 to 20 hours. They were then weighed, placed under a bell jar and anesthetized with ether. Following the induction stages, the anesthesia was continued by the cone drop method. After preliminary sampling and injection of the dye, the right femoral artery was cannulated and connected to a Hürthle manometer. In one experiment (no. 9), through the kindness of Dr. H. Wiggers, blood pressure and pulse variations were recorded by means of an optical manometer. The attempt was made to regulate the depth of anesthesia and the heat exchange of the animal in such a way that blood pressure, respiration and

rectal temperature remained fairly constant during the hour or more of intermittent sampling prior to the transection of the cord. Difficulties with clotting experienced in preliminary experiments were met by using a

TABLE 1

Effects of cord destruction at C_8 in acute experiments under ether anesthesia

CAT	SEX	WEIGHT	HEMATOCRIT, PER CENT CELLS			MEAN ART. PRESSURE, MM. Hg			SERUM PROTEINS, MGM. PER CENT			PLASMA VOLUME, CC.			REMARKS
			Control	Postop.	Diff.	Control	Postop.	Diff.	Control	Postop.	Diff.	Control	Postop.	Diff.	
		kgm.													
1	♂	2.93	39.4	37.2	−2.2				6.3	6.0	−0.3	138	147	+9	Animal intended for survival
2	♂	3.96	27.0	24.9	−2.1	145	73	−72	6.2	5.9	−0.3				Lipemic serum
3	♂	2.92	31.7	30.5	−1.2	140	80	−60	6.3	6.0	−0.3	110	116	+6	
4	♀	3.13	39.6	37.1	−2.5	130	85	−45	6.4	6.4	0	119	128	+9	
5	♂	3.13	38.2	32.8	−5.4	115	75	−40	6.8	6.4	−0.4	148	154	+6	
6	♂	3.19	41.3	37.8	−3.5	160	120	−40	6.0	5.6	−0.4	118	122	+4	
7	♂	3.43	29	29	0	145	85	−60	6.0	6.0	0	151	151	0	
8	♂	3.52	43.2	38.7	−4.5	155	84	−71	5.9	5.3	−0.6	111	124	+13	
9	♂					175	95	−80							With optical manom.
Average					−2.67			−58.5			−0.29			+6.7	
Average as per cent of control					−7.79			−42.2			−6.7			+5.5	
10	♀	2.75	45.8	43	−2.8	105	60	−45	5.8	5.6	−0.2	115	123	+8	Atropinized
11	♀	2.59	35.5	29.2	−6.3	125	70	−55	6.5	6.1	−0.4	105	105	0	Atropinized
12	♀	2.36	28.7	26.4	−2.3	125	85	−40	6.8	6.8	0	141	141	0	Atropinized (Lactating and feverish)
13	♂	2.69	36.7	34.2	−2.5	120	75	−45	7.1	6.9	−0.2	138	142	+4	Atropinized
14	♀	2.36	48.1	44.8	−3.3	140	100	−40	6.1	5.9	−0.2	106	111	+5	Atropinized
15	♀	2.46	40.9	38.8	−2.1	130	130	0	7.0	7.0	0	93	93	0	Vagotomized
16	♂	3.70	42.6	38.9	−3.7	140	72	−68	6.8	6.1	−0.7	161	170	+9	Vagotomized
17	♂	3.30	44.4	40.3	−4.1	170	72	−98	6.6	6.0	−0.6	101	114	+13	Vagotomized
18	♀	3.21	35.2	33.5	−1.7	155	72	−83	6.1	5.9	−0.2	129	132	+3	Vagotomized (Pregnant)
19	♀					140	105	−35							
Average					−3.20			−50.9			−0.28			+4.7	
Average as per cent of control					−7.99			−31.2			−3.5			+3.4	

cannula of large bore, carefully coated with paraffin. If care was taken to avoid chilling the arteries with cold instruments, good sized cannulae could be introduced even into the arteries of small cats, and once the skin wound was closed with a clamp, a continuous record could be secured for long periods without clots.

Upon the destruction of the cord segment, the use of the anesthetic was discontinued except for brief control periods occasionally introduced after sampling had been completed, in order to demonstrate that ether withdrawal had not caused the observed change in blood pressure. A few experiments upon chronic spinal cats were also undertaken, in order to determine the effect of the administration and withdrawal of ether upon the plasma volume of such animals. The results are summarized in table 2. In some of the acute experiments, bilateral cervical vagotomy was performed shortly before the first dye sample was taken. In others, atropine was given intraperitoneally a half-hour before the induction of anesthesia. The dosage was such as to produce mild mydriasis, generally 0.04 mgm. per kgm. body weight.

TABLE 2

Effects of ether anesthesia upon chronic spinal cats

CAT	SEX	WEIGHT	DAYS SPINAL	DURATION OF ANESTHESIA	HEMATOCRIT, PER CENT CELLS			SERUM PROTEINS, MGM. PER CENT			PLASMA VOLUME, CC.			REMARKS
					Control	Ether	Diff.	Control	Ether	Diff.	Control	Ether	Diff.	
		kgm.		*min.*										
20	♂	2.27	4	89	33.7	34.6	+0.9	5.1	5.4	+0.3	133	120	−13	Excessive salivation
21	♂	3.14	11	64	30.4	27.7	−2.7	6.8	6.7	−0.1	167	167	0	
22	♂	2.39	6	72	30.4	27	−3	6.0	6.1	+0.1			0?	Plasma volume curve indecisive
23	♂	2.62	63	44	34.0	26.7	−7.3	6.9	6.8	−0.1	138	138	0	
1	♂	2.51	42	50	32.0	32.5	+0.5	6.7	6.1	−0.6	152	169	+17	
20	♂	2.10	28	54	30.0	24.5	−5.5	5.6	5.7	+0.1	135	135	0	Atropinized
23	♂	2.50	55	48	33.0	33.8	+0.8	6.4	6.4	0	114	114	0	Atropinized
1	♂	2.50	56	40	24.9	29.1	+4.2	6.7	6.4	−0.3	149	158	+9	Atropinized

RESULTS. The principal results are summarized in tables 1 and 2. The general circulatory changes which are usually associated with high spinal transections were observed with regularity. During the destruction of the cord segment, the mean femoral pressure underwent a transient rise which was sometimes very great and sometimes barely perceptible. In all experiments except one (no. 15), this initial rise was succeeded by a fall, the amount of which, in cats with intact vagi, averaged 42 per cent of the control value, and in vagotomized cats, 37 per cent of the control value. In most instances the minimum pressure was reached within five minutes after the operation, and was followed by partial recovery. In the animals with cut vagi, the blood pressure regained as much as two-thirds of the control value within one hour following the transection. When partial recovery occurred in the non-vagotomized cats, the extent was much less

in a corresponding interval. Previous section of the vagi also appeared to diminish the time required for recovery from ether.

During the primary rise in pressure which frequently attended the destruction of the cord segment, the heart rate was quickened. The increased rate was maintained throughout the falling phase of the pressure curve, during which time, also, the pulse pressure was usually much reduced. In many experiments transient cardiac arrhythmia appeared during the time of initial high pressure, but this vanished before the minimum pressure level was attained. Following the period of tachycardia, the heart rate gradually fell, and during most of the recovery period it averaged 15 to 20 per cent lower than the control value. With the exception of cat 15, this secondary slowing of the pulse occurred in the vagotomized animals as well as in the others. Cat 15 maintained an increased heart rate throughout the period of observation, and entirely escaped the fall in pressure, the only significant change being a moderate and fleeting rise as the cord was attacked.

The plasma volume changes were neither profound nor prolonged. As indicated in table 1, the maximum deviation in any one experiment was never more than 13 per cent, and in many experiments the observed changes are well within the 5 per cent limit of experimental error generally conceded for the dye method. The nature of the technic, however, is such that systematic error is not likely to affect the essential results, and inasmuch as all of these indicate a change in the same direction, the data may be of some significance even though the values concerned are small. The dilution of the plasma proteins is in fairly good agreement with the dye estimates. Since only two hematocrit measurements were made in each experiment, less reliance can be placed upon these values. As the figures stand, however, they seem to indicate a dilution of the blood of the same order of magnitude as would appear from the dye and protein measurements. Of the twelve animals in which a significant change in plasma volume occurred, three showed recovery to the control level within sixty to eighty minutes. In the same interval, two cats showed an additional concentration beyond the control level, five showed partial recovery, and two showed no recovery at all. A graph of one experiment is given in figure 3.

Table 2 shows the results of ether inhalation by chronic spinal cats. These experiments are of value in the interpretation of the results described above. Neither upon the administration nor upon the withdrawal of ether was any change encountered of the sort that followed the acute transections. In several instances, the induction of anesthesia was accompanied by profound vagal effects, including temporary cardiac asystole and cessation of respiration.

Discussion. The experiments reported here show that in cats under ether anesthesia, low blood pressure following cord transection is usually

accompanied by a small increase in the volume of plasma. The results of the administration of ether to chronic spinal cats make it seem improbable that the plasma volume changes found in the acute procedures are directly related to the state of narcosis. Such a relation is made still less likely, although it is by no means excluded, by Conley's observation (1) that the

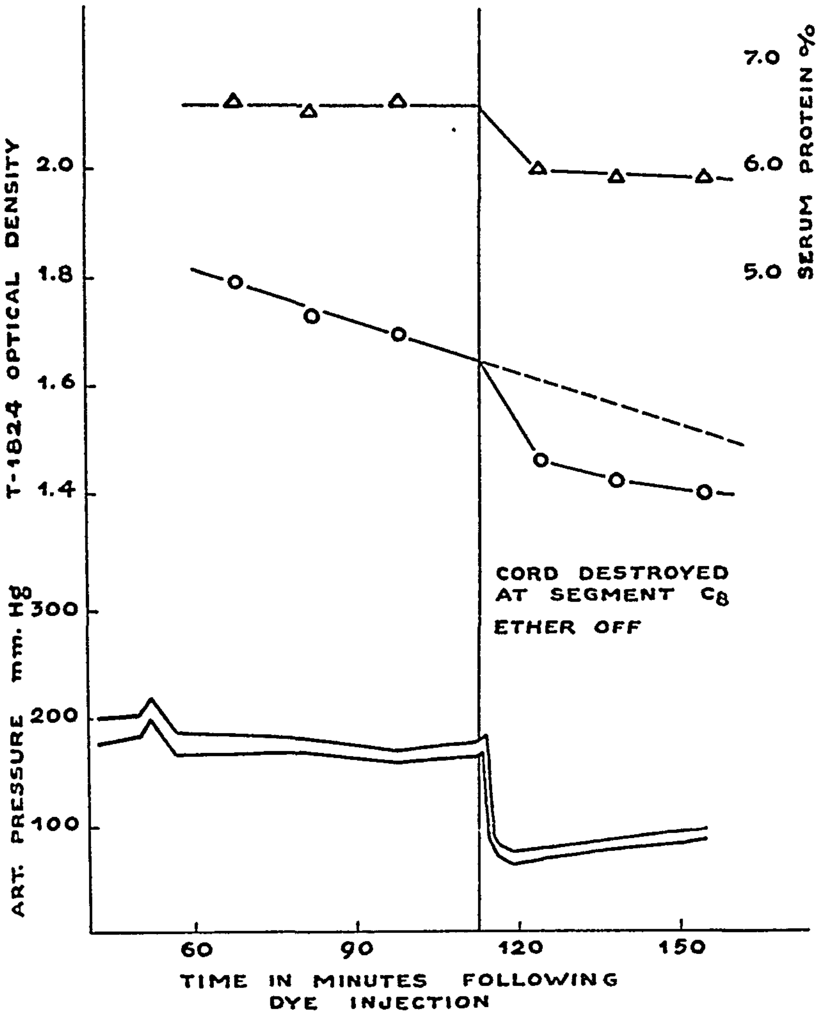

Fig. 3. Graph of experiment 17. Adult male cat with both vagi cut in neck. For photograph of cord lesion, see figure 1.

administration of ether to normal cats is without effect upon the plasma volume. Hamlin and Gregersen (5) found that in cats in which vasoconstrictor pathways are interrupted by total sympathectomy, rather than by cord section as in the present experiments, the volume of the circulating fluid may undergo a large increase.

Various observations indicate that a parallel does not necessarily exist between plasma concentration and arterial pressure reduction. Root and

McAllister (11), for example, showed that inhalation of ether by spinal or sympathectomized dogs results in a fall in blood pressure without any change in plasma volume. In the surgical stage of ether anesthesia, normal dogs exhibited little change in blood pressure, and a consistent reduction of plasma volume (8). The arterial pressure is reported to be reduced by ether in spinal cats as it is in spinal dogs (12), but the data given in table 2 reveal no significant change in plasma volume under these circumstances. The present results, together with those cited above, emphasize the fact that plasma volume changes cannot be predicted with certainty from changes in the arterial pressure alone.

Thanks are due to Dr. Magnus I. Gregersen and to Dr. Walter S. Root for suggesting this problem, and for constant help and encouragement.

SUMMARY

1. A method is described for rapid and relatively bloodless destruction of a segment of the cervical cord of cats by blind puncture and cauterization.

2. Acute destruction of the eighth cervical segment under ether anesthesia was followed by a fall in arterial pressure averaging about 40 per cent, and by an increase in plasma volume averaging 5 to 6 per cent (fig. 3 and table 1).

3. Previous atropinization or bilateral vagotomy did not significantly affect these changes (table 1).

4. Administration of ether to chronic spinal cats resulted in no consistent change in plasma volume (table 2).

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THE SPREAD OF EXCITATION IN TURTLE, DOG, CAT AND MONKEY VENTRICLES[1]

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The sequence of excitation of the various areas of the ventricular surface of the hearts of experimental animals, mainly dogs and turtles, has been investigated many times and by a variety of methods of leading to electro-registering instruments (18, Chapter I, Methods and Bibliography).

The unipolar method in different hands has yielded results which disagree significantly (15, 19, 1). The validity of the monophasic method employed by H. C. Wiggers (21) has been challenged, since it has been found that an injured area becomes positive upon activity of the surrounding myocardium (8, 17). Differential electrodes used by Clement (3) and by Erfmann (6) have a high electrical resistance, and have been found in our laboratory to yield no deflection in string galvanometer records when arranged to make a small contact and with a string tension that was considered permissible. Coupled to the string galvanometer or cathode ray oscillograph through a suitable amplifier, differential electrodes have recently found considerable use in the study of the relation between local contraction and electrical events (12, 10). The main peak of the differential curve occurs approximately at the moment of contraction and coincides with some point on the steep ascent of negativity in corresponding unipolar curves.

The lack of agreement as to the temporal spread of the excitatory process, and even of the electrical sign indicating its occurrence in unipolar and differential records appears to justify a re-examination of the problem by a different approach. In addition to the experiments on the ventricles of turtles and dogs the study was extended to include the ventricles of cats and monkeys.

Recording methods. Three large Hindle string galvanometers were arranged to record simultaneously. By means of two mirrors and a shield the central parts of all three light fields were thrown side by side on the face of the camera, approximately perpendicular to it, and without overlapping. Projection distance from each was one meter. A constant speed

[1] This research was supported by a grant from the John and Mary R. Markle Foundation.

rotating wheel with vanes long enough to interrupt the light across the face of the camera provided simultaneous reference lines 40 msec. apart across the whole width of the paper. With the aid of the vertical lines, a binocular magnifier and a 3 msec. scale recorded at the speed of the camera, measurements could be made accurate to within 1 msec. deviation. A large comparator was used on a few records, but it was cumbersome and added nothing to the accuracy of measurement. One galvanometer recorded an electrocardiogram or other reference record, and the others, electrograms from the ventricles. For external surface leads electrodes were desired which could be used with the string galvanometer to record action currents of local origin excluding influences from distant sources to the greatest practicable degree. The high resistance of fluid electrodes and the instability of wicks rendered the differential electrodes of Clement (3) unsuitable for the purpose, though they have the advantage of localized leading. To retain, and possibly improve upon, this property of localization, and eliminate the handicaps mentioned, a new type of electrode assembly appropriately called *riding contiguous bipolar* electrodes was devised. The word *contiguous* is used here in the sense of points that are *very near together but not in contact.* These electrodes consisted of two pieces of 0.035 inch silver wire mounted in a small lucite block (fig. 1). The silver pieces extended about 8 mm. out of the block, and during experiments the ends were close enough together that the overall span of the contact of

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Fig. 1. Details of the riding contiguous electrodes. *S*, the silver wires; *J*, jackets; *B*, lucite block; *W*, flexible resilient wires; *L*, supporting lead pipe.

Fig. 2. Records from turtle's heart. Upper electrogram, from contiguous electrodes. Middle electrogram, from unipolar leads. Lower record, R of reference electrocardiogram; 1 and 2, the two slopes of R. Intervals, vertical lines 40 msec.

Fig. 3. Upper electrogram, from external surface of turtle ventricle near apex. Middle electrogram from internal surface immediately opposite external lead. Lower record, reference electrocardiogram. Intervals, 40 msec.

Fig. 4. Shapes of unipolar records from different parts of the turtle's ventricle, and the constancy of relation of the onset of the extrinsic portion to the more gradual slope of the reference R. Intervals, tuning fork, 20 msec. Upper electrograms: A, from early area near left base; B, near left mid-ventricle; C, later area just to right of apex. Lower electrograms; R waves from lead II.

Fig. 6. Records from the dog's ventricle. A, B, C and D, upper electrogram, contiguous electrode record; middle, unipolar record, one of the contiguous contacts serving as the stigmatic pole to the unipolar galvanometer; bottom electrogram is from a reference contiguous electrode in the central area. Exploring leads unmoved between A and B, and between C and D.

A and B, from early central area; C and D, from relatively late area near conus. Note small pre-R elevation in C.

E, upper electrogram from point on external surface near base of right ventricle; middle electrogram on internal surface immediately adjacent to external lead. Lower record, reference contiguous electrogram. Intervals, vertical lines 40 msec.

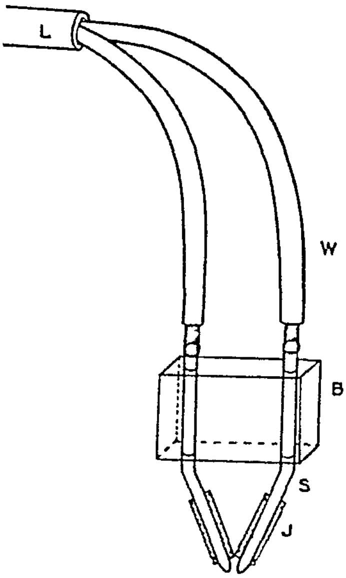

FIG. 1

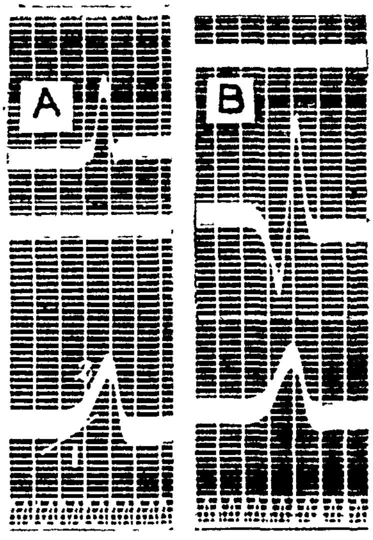

FIG. 2

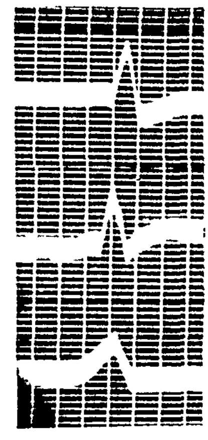

FIG. 3

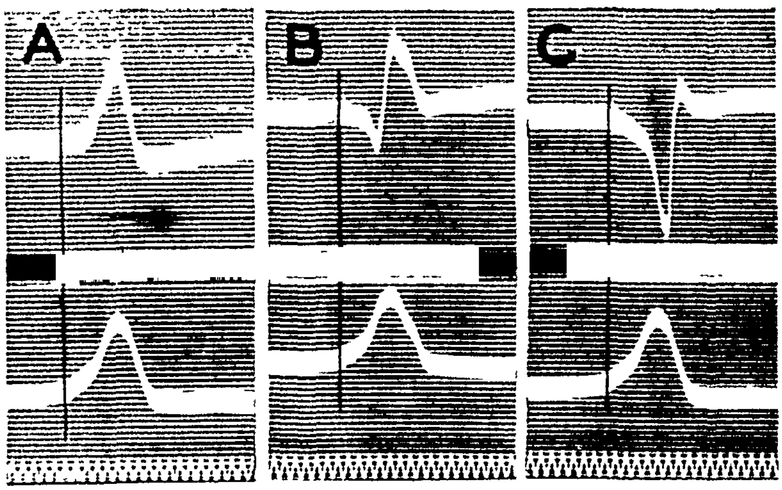

FIG. 4

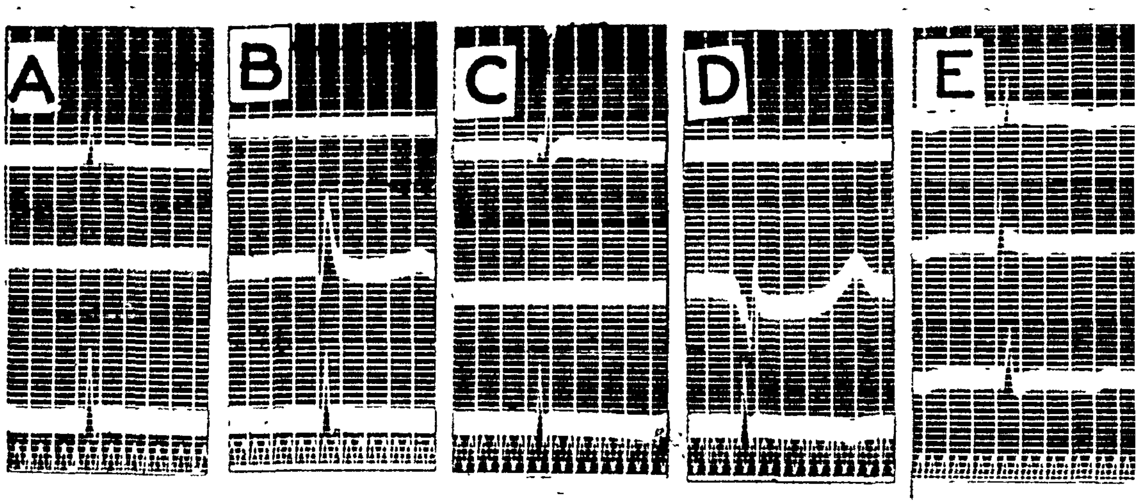

FIG. 6

their tips with the heart was about $1\frac{1}{2}$ mm. The ends should not be sharp, but a long rounded contour helps them maintain their position on a given locus of the surface of the beating heart, and reduces the width of their span. The wires which connect these electrodes with the control box serve also as a resilient suspension for the assembly. This provides for the "riding" property which is essential to the maintenance of a constant contact with the moving ventricle. Wires from discarded Harvard platinum electrodes with all but the last layer of insulation stripped away, and with $1\frac{1}{2}$ to 3 inches extending out of the supporting lead tube were found to permit the electrodes to follow the heart and yet be not too limber. In some experiments with dogs and turtles, records were made from the internal surface of the ventricle by leading with bipolar silver electrodes at the ends of suitably curved insulated tubes inserted via an auricle.

The method of chloriding electrodes is important. Silver electrodes prepared by the Langelaan method (13) were found unsatisfactory for cardiac leads, but a series of experiments yielded a method which produces a high degree of non-polarizability which lasts well under experimental conditions. The silver is cleaned with strips of fine abrasive paper, then immersed to the desired depth in 5 to 10 per cent NaCl and attached to the positive pole of a 3 volt battery. The circuit is completed by a silver or platinum foil electrode attached to the negative pole and immersed. After one minute the connector is moved to the $1\frac{1}{2}$ volt battery post and the process is allowed to continue for 20 minutes longer. After preparation, the electrodes are kept wet with physiological saline.

In use it was found that electrodes which showed no polarization when immersed 3 or 4 mm. in Ringer's solution would polarize perceptibly when removed from the fluid and the ends placed in contact with the heart. In the latter case the area of the electrode-saline interface was very small. When this interface was enlarged by placing a jacket saturated with saline solution around each of the silver pieces the non-polarizable quality was greatly improved. The jackets are practically indispensable also because they maintain a constant level of contact between fluid and electrode, thus preventing potential changes by shifting of fluid levels as the heart moves. Jackets should be removed during cleaning and chloriding of electrodes. A segment from the woven tubular outer layer of a small round shoelace serves well as a jacket.

On theoretical grounds the contiguous bipolar, like the differential electrode, should minimize the recording of potentials of extrinsic origin. Reference to one of the several published diagrams (e.g., 11, fig. 2) showing the distribution of isopotential lines about two oppositely charged points in a plane conductor, or about the active-inactive boundary in tissue makes it clear that closely paired points not in close proximity to the source of potential differences will be practically equally affected. This is not true

if the two leads are far apart, or if one of the pair is remote. Practical tests support these deductions. Contiguous electrode records from the dog's auricles show no ventricular deflection, and ventricular records show no auricular influence. Electrodes on the dog's aorta 2 mm. from the myocardium show no deflection. The freedom from extraneous electrical influences was also demonstrated by applying short D.C. potentials of more than 40 volts through electrodes 1 cm. apart and less than 1 cm. from the leads, without causing excessive movements of the string. In a sample experiment, the recorded potential difference was 0.05 per cent of that applied 1 cm. away. By careful orientation this can be even further reduced. It, therefore, appears safe to conclude that any deflection in the riding contiguous electrode record is the result of electrical activity within a local area of small dimensions.

Rotation of the electrodes, maintaining the same locus as exactly as possible, makes large changes in the spike recorded, but none or very little in the moment of its beginning. The changes are in height, width, and direction. The recorded potential difference (height) varies from maximal to minimal with changes in orientation, but the changes are not entirely predictable. The variation in separation of the limbs ranges (e.g., on the dog's ventricle) from the narrowest that the string can record to a maximum of about 18 msec. These changes cannot be accounted for in terms of conduction along the surface alone, but must involve conduction to the surface fibers. Insufficient information is available concerning the detailed relationships of conduction in the various cardiac tissues to afford a clear explanation at present.

When polarizing currents or brief stimuli evoke idioventricular contractions, much greater changes than those produced by rotation of the electrodes may be seen. The deflections become wider and may assume bizarre shapes. This is especially true at points very near a polarizing electrode, or in discharges resulting from stimuli delivered in late systole, or in multiple trains of discharges from such a stimulus. These changes are due to alterations in rate of conduction and of the direction at which impulses approach the two electrodes. These observations suggest new uses for such electrodes in the study of conduction and its disturbances and alterations.

Turtles. Specimens of *Pseudemys elegans* with carapace width of 5½ to 6 inches were used. Exposure without bleeding was made by trephine opening (3 in. in diameter) in the plastron over the heart region. The leads for the reference electrocardiogram were freshly prepared large silver-silver chloride wires, one of which was embedded in saline saturated cotton just cephalad to the auricles and about 2 cm. to the right of the midline. The other was inserted in the muscle in the left side of the pubic region. Once placed, these electrodes remained undisturbed throughout the experi-

ment. In order to compare the contiguous electrode and unipolar methods of recording and to more surely recognize the local electrical sign in both kinds of records, many experiments were done in which the two kinds of records were made from the same place, one of the contiguous contacts serving also as the active unipolar lead. In such cases records were made from each galvanometer with the other turned off because intercoupling occurred when both of these galvanometers were recorded simultaneously. The electrodes were not moved between these determinations. In records from turtles the sharp onset of the spike in the contiguous electrode record usually coincides with onset of the negative upswing of unipolar electrode records within 5 msec. Ordinarily, the coincidence is closer, frequently exact. Rarely, there are splintered, unusual shaped deflections in unipolar leads that cannot be used.

The relationship of unipolar and bipolar leads to R of the reference electrocardiogram is shown in figure 2. The reference deflection exhibits two slopes marked 1 and 2 (shown also in fig. 4). The examination of many records disclosed that no area on the surface of the turtle ventricle exhibits signs of surface excitation (sharp onset of contiguous electrode record or negative upswing in unipolar record) earlier than about the moment of the bend from the more gradual to the steeper slope of the reference R. The earliest surface records are approximately simultaneous with this bend and excitation at all later areas comes on the steep slope to the peak and beyond the peak. Deflections recorded from the internal surface of the ventricle have been found to precede those from adjacent external areas by 15 to 40 msec., and they occur mainly within the interval occupied by the more gradual slope of R, though from late areas both spikes may occur during the steeper slope (fig. 3).

As shown in figure 2A, the contiguous electrode spike is sometimes preceded by a slight deviation just ahead of the sharp break. This is not synchronous with any feature of the reference electrocardiogram. It is probably of subsurface origin, and does not at all obscure the onset of the spike. In contrast to the deflection recorded by contiguous electrodes which upon correct orientation always display clear spikes, records from unipolar leads on different parts of the ventricle are differently shaped. From the areas that exhibit early surface spikes in contiguous leads the initial deflection (R complex) of the unipolar lead is entirely in the negative direction, the surface excitation being indicated by an abrupt rise of a steep negative slope from one that was low and flat. In areas that show surface excitation latest the deflection is wholly or almost wholly below the base line, movement in the negative direction being chiefly a swift return. Between these there are many gradations corresponding to the relative times of surface excitation (fig. 4). In all cases the positive phase, or the low negative extrinsic phase in regions without a positive phase

begin simultaneously with the more gradual slope of R of the reference electrocardiogram (fig. 4). There is no progression in the onset of the extrinsic phase of unipolar records from the surface of the turtle heart.

RESULTS. The sequence of excitation of surface areas as derived by the methods described is in general agreement with reports that have been made from studies on other species of turtles (14, 16, 12). Figure 5 shows the results of an experiment that may be considered as typical during the spring. The interval between earliest and latest points is 34 msec., and is usually within a few milliseconds of 40. During the winter this interval may be 60 to 70 msec. In late summer it is much less, frequently in the neighborhood of 25. The sequence, however, remains relatively the same. The earliest points are in the left basal and left central areas, and the direction of spread is somewhat diagonally toward the apex and toward the right. The latest point is frequently on the right border no more than halfway from base to apex.

Dogs. Dogs were anesthetized with morphine and barbital sodium. The chest was opened along the sternal midline and the heart suspended in a pericardial cradle. The ventricles of ten dogs were studied. Three pairs of leads could be applied to the ventricle simultaneously. In some experiments they were all contiguous sets, one for a constant reference record and two for punctate measurements.

RESULTS. Records from contiguous electrodes consist of an initial complex and a T wave (fig. 6). The initial complex has a prominent sharp spike deflection R, and from some areas, one or more small (pre-R) deflections, shown in figure 6, C. The first pre-R event may precede the main spike by any interval from 0 to 20 milliseconds. In some records it is merely a bevel of 1 to 3 msec. leading to the sharply breaking upturn of R, and in others no preceding event is detectable. The interval between pre-R and R is smallest in the thin-walled central and trabeculated areas of the right ventricle. Records from various points on the right ventricle made with internal and external leads opposite each other and separated only by the thickness of the myocardium show that the deflection from the internal lead (fig. 6, E) always preceded the external deflection. In the thin central portion where early surface responses occur, the interval of internal precedence ranges from 1 to 5 msec., while over the rest of the right ventricle, sampling all parts except the conus, the difference was 9 to 12 msec. The range of interpunctate intervals on the endocardial surface is small. The maximum in the right ventricle is about 6 or 7 msec. The internal spike appears to have a close association with the pre-R surface events, but they are not identical. When the pre-R elevations were multiple the internal spike occurred during one of them; when single, they appeared simultaneously, or the internal spike fell within the duration of the pre-R deflection. The internal spike usually was narrow and

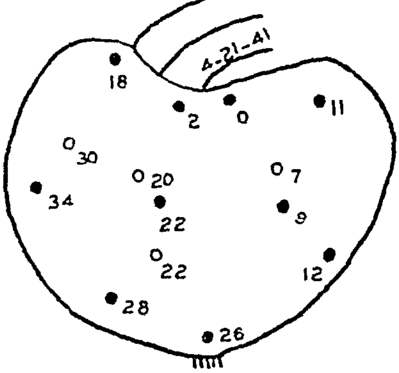

FIG. 5

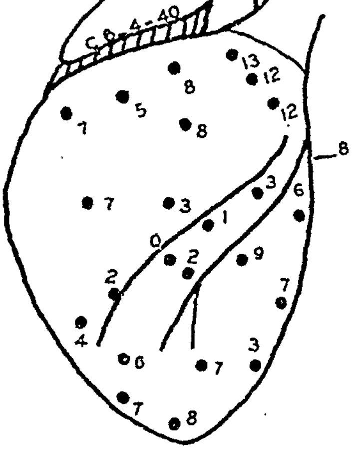

FIG. 8

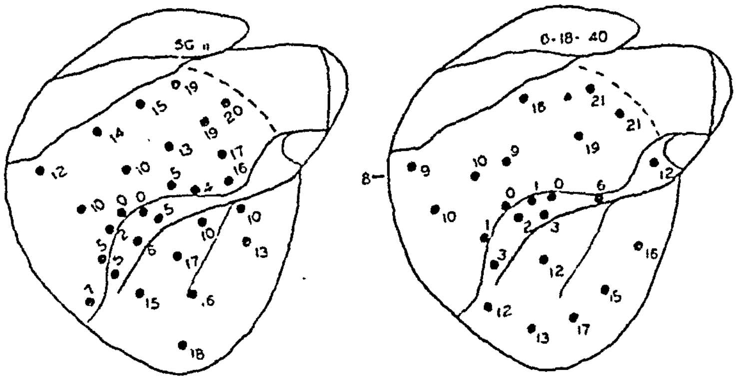

FIG. 7

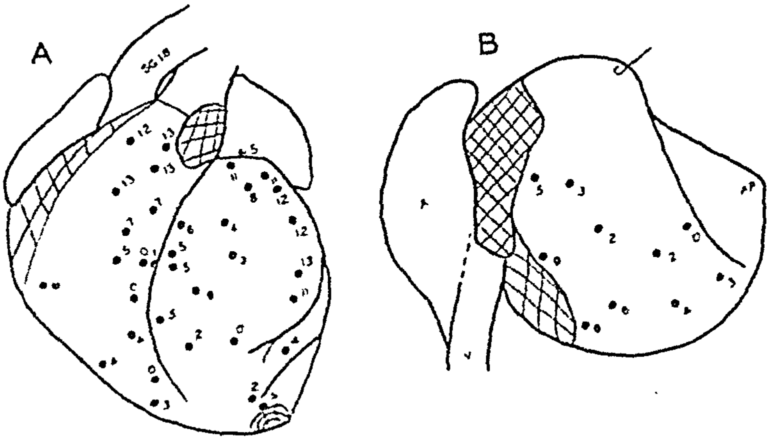

FIG. 9

Fig. 5. Surface excitation of the turtle heart. Solid dots, ventral surface; circles dorsal surface. Figures show intervals in milliseconds after excitation of the earliest point recorded. Spring experiment: April 21st.

Fig. 7. Surface excitation of the dog's heart. Two experiments. Figures, milliseconds after earliest point recorded.

Fig. 8. Surface excitation of the cat's heart. Sample experiment. Figures, milliseconds after earliest point recorded.

Fig. 9. Surface excitation of monkey's heart. A, ventral surface, and extending a considerable distance on the dorsum of the left ventricle. B, heart drawn far over to expose the side and dorsal aspects of the right ventricle. Figures, milliseconds after earliest point recorded. Cross-hatched areas are fat pads.

sharp. The pre-R deflections probably are not records of action currents in the subendocardial conducting tissues themselves, but they are associated with conduction beneath the external surface of the ventricle.

The relations of bipolar and unipolar leads to a standard bipolar lead from another area are shown by comparison of A, B and C, D of figure 6. Unipolar records contain a positive phase followed by a returning upswing which continues beyond the base line as a negative wave (fig. 6 B). The relative prominence of these two vary with the time of excitation of the areas, much as in the case of the turtle ventricle (cf. fig. 6, B and D). These measurements were made by contiguous and unipolar leads from the same placement of electrodes, one of the contiguous points serving as the stigmatic pole of the unipolar pair. As in the turtle experiments, the galvanometer connected to the unipolar leads was turned off (infinite resistance), while the contiguous electrode record was being made and vice versa, but the electrodes were not moved between records. The deflections in all leads are much more consistently sharp and unequivocal from mammalian hearts than from the turtle. It is exceedingly rare that one has cause to question the instant of onset of the contiguous electrode spike within 1 msec. and the unipolar upstroke in the negative direction is usually free of complication. Occasionally there is an angulation of five to ten milliseconds' duration, ending in a sharp bend upward. In all cases the onset of the contiguous spike and the sharply upbreaking point of the unipolar deflection coincide most closely, rarely varying from each other by more than 2 msec. Neither event bears any significant relation to the onset of the positive phase of the unipolar record.

Figure 7 shows the relative times of surface excitation at points on the ventral aspect of the right and left ventricles in two dog experiments which may be considered typical. As previously reported by others (15, 19), the earliest points are found in the central area on the trabeculated region of the right ventricle and near mid-septum. The process arrives at other areas of the right and left ventricles later, the conus latest of all, though in some hearts it is not excited much later than some points on the left ventricle. A few records made from the vortex were found to be quite early, 4 to 7 msec. after the earliest point. In no experiment was the span between earliest and latest points on the ventricular surfaces more than 22 msec., with a variation from 18 to 22. These figures considered in conjunction with those derived by internal leading and internal-external pairs tend to confirm the deductions of Lewis and Rothschild (15) that the impulse arrives at all parts of the endocardium within a very brief interval and from there proceeds through the myocardium to the surface much more slowly.

Cats. The hearts of eight cats were studied. These animals were anes-

thetized with dial[2] and the thoracic contents exposed by midsternal sagittal opening in a manner generally similar to that used in the dog. The ventricles were explored with the contiguous electrodes and the relative excitation times plotted. As in the dog ventricles, there is a degree of orderly sequence, but with some variations in absolute values found for corresponding points in different hearts. Figure 8 shows the results of one experiment which may be considered as a fair sample. Like the dog ventricles, the earliest area is near mid-septum and in the immediately neighboring region of the right ventricle. The relationships in other regions resemble those of the dog heart also, but the values are smaller, generally one-half to two-thirds of the figure for the corresponding region of the dog ventricle. In some cat experiments, one difference was noted. In regions along the margin of the left ventricle ordinarily exhibiting an action current 6 to 8 msec. after the earliest there would be an occasional reading that was very early, only 1 or 2 msec. after the earliest. The meaning of this has not been learned. Erfmann's (6) measurements on cats' hearts with differential electrodes were chiefly from the base and apex. Some of his base-apex intervals are greater than any found in these experiments. Surface contiguous electrograms from cats and monkeys show pre-R elevations qualitatively like those in dog records.

Monkeys. Two hearts of Macacus rhesus monkeys were mapped, many points being recorded from both dorsal and ventral surfaces. The results of one of these experiments are shown in figure 9. The pattern of surface excitation appears similar to those of the dog and cat with respect to certain large features, but there are also differences which may be significant. Similarities are points of earliest excitation in the central region of the right ventricle near the septum, and late areas on the conus region of the right ventricle and at the more basal and dorsal portions of the left, the latter being excited relatively later in the monkey's heart. The most striking difference is in the irregular and widespread area which receives excitation early, as shown in figure 9A. The whole right ventricle, except the conus region and a basal rim band extending far around on the dorsal aspect is excited within 5 msec. In the experiment illustrated in figure 9A, the area of early excitation also includes the apex and right half of the left ventricle, though there are points immediately left of the septum which are later than apical and mid-ventral portions of the left ventricle. Measurements on the two hearts agreed closely except on the latter two areas, the apex and mid-ventricle on the heart not illustrated showing values of 6 and 7 msec. after the earliest. The breadths of span from earliest to latest points were 13 and 14 msec. in the two hearts.

[2] The dial used in the experiments on cats and monkeys was generously contributed by the Ciba Company, Inc.

DISCUSSION. A consideration of the fundamental nature of excitation or of action potentials is not the purpose of this presentation, but in order to measure the temporal spacing of events one must be able to recognize their signs. It has been seen that the onset of the portion (positive or negative) of the unipolar complex preceding the quick upstroke led from any part of the ventricle coincides in time with the beginning of the more gradual slope of R of the electrocardiogram which is temporally associated with excitation and conduction in tissues within the turtle ventricle (perhaps the auricular funnel tissue). Therefore, this part of the unipolar record can be dismissed as being no part of the electrical manifestation of local surface excitation. The close agreement between the onset of the spike in the contiguous electrode record and the steep upstroke of the unipolar record supports strongly the old idea that the sharp movement in the negative direction is the unipolar manifestation of an action current in the local surface area. This finding agrees not only with the great mass of observations from complex tissues but also those of recent experiments in which excitation has been studied under conditions of minimal anatomical complication. When electrodes were placed opposite each other, one on the inside and the other on the outside of the membrane of the squid giant axon the action potential with reference to the outside lead consists of a negative spike and an after-potential (5). It has recently been demonstrated that the large decrease in electrical impedance (depolarization?) across the membrane of the squid giant axon and of Nitella which accompanies excitation by a propagated impulse occurs during the rising phase (negative) of the monophasic action potential. The part of the action potential preceding the local membrane changes is tentatively interpreted as a passive fall of potential affecting the electrode ahead of the approaching partial short circuit (4). If this explanation is correct, action potential leads sufficiently near together should tend to eliminate the preceding fraction and approach the limit of zero interval between the onset of the action potential and the fall of impedance. Bishop (2) has referred to studies of electrical phenomena occurring on excitation of plant and animal cells, citing them as evidence that the process of excitation is general.

The variations in the interval found between excitation of earliest and latest external surface points on the turtle ventricle agree quite well with those of Lewis (14) if both summer and winter experiments are included.

The results of measurements on the dog's ventricles agree quite closely with some of those of Lewis and Rothschild (15) and of C. J. Wiggers (19). The same general sequential pattern exists and frequently there is close quantitative agreement. The main difference found is in the magnitude of the variations reported in some of their experiments. Instead of an interval of 18 to 22 msec. between earliest and latest points in different

experiments and a maximal variation of 4 or 5 msec. between corresponding points, they report wider variations. In the experiments of Lewis and Rothschild the interval between earliest and latest points was nearly always greater than 20 msec. and frequently approached 30. C. J. Wiggers' variations were from less than 20 msec. to more than 30. Doctor Wiggers has expressed the opinion that the stitching of the wick lead to the myocardium, though done in many experiments with an oiled horse-hair, may have affected the records.

The experiments with cats may offer a clue to some of the variations seen in experiments on dogs. The cats' hearts used were much smaller than those of the dogs, but the excitation pattern was quite similar. The differences are quantitative and appear to obey a fairly constant proportionality throughout the various areas. This suggests that the differences in intervals noted between cats' hearts and dogs' hearts may be a function of size. Our dogs were of fairly uniform weight, 9 to 12 kgm., and therefore do not give a conclusive answer. It appears possible, too, that some of the largest figures of others for interpunctate intervals were derived from very large hearts.

The results reported by Abramson and Jochim (1) were obtained with the exposed thoracic contents covered by a warm moist chamber. The figures which they chose as the true ones showed maximal interpunctate intervals on the ventral surface of the dog's ventricle of no more than 8 to 9 msec. They attribute the greater differences in the results of Lewis to surface cooling and drying of the exposed heart. The factor of drying has been prevented in our experiments by meticulous care to keep the ventricle moist by an automatic dropping system. To test the "cooling hypothesis" the surface temperature of a ventricle which had been used with the chest open for six and one-half hours and which had been fibrillated and defibrillated a number of times was measured with a Tycos dermatherm with the stigmatic junctions of the thermopile covered with cellophane. The rectal temperature was 37.2°C., the room temperature 26, and that of the ventricular surface, 37.0. This is in confirmation of a series of measurements by Wiggers and Wegria (20) who found that the temperature of the cardiac surface in open-chested preparations closely approximates that of the blood. The results of Abramson and Jochim need a different explanation and confirmation.

The experiments of Erfmann (6) with differential electrodes are sometimes cited as evidence that the various areas of the dog's ventricle are excited simultaneously. But Erfmann's protocols show interpunctate intervals which might be considered large, e.g., in experiment IX, base-apex differences range from 6 to 18 msec. The largest of these rank among the highest of comparable values reported by Lewis and Rothschild or C. J. Wiggers. The experiments of H. C. Wiggers (21) using the mono-

phasic method of recording showed exactly simultaneous moments of onset of the monophasic curve over large ventricular areas, and then other series whose values differ from the first group, but are simultaneous within each areal grouping. Such groupings appear to be in accord with the statement of Eyster, Meek, Goldberg and Bartsch (9) that the monophasic method measures the moment of onset of activity in areas adjacent to the injury rather than in loci to which the "active" electrode is applied.

Eyster, Meek and Gilson (7) measured the sequence of occurrence of differential peaks from loci distributed over the dog's ventricle. The information in their brief report is consistent with the measurements of onsets of contiguous electrode spikes or unipolar negativity, but only gross deviations would be subject to detection. In view of the findings of Cole and Curtis, it is possible that the peak of the differential curve may offer a fair approximation to the moment of impedance fall. However, in consideration of the fact that rotation of contiguous electrodes changes the width of the spike and the temporal distance of the peak from the onset, the onset may be expected to bear a more constant relation to the moment of the first local excitation change under the earlier of the lead points than does the peak.

SUMMARY

Methods of electrographic leading have been considered and a new type of simple leading electrode assembly, riding contiguous electrodes, described. Evidences are offered to show that cardiac action currents recorded by them are local in origin.

Two slopes are described in the electrocardiographic R wave of the turtle. The earlier, more gradual one, occurs during the time that local spikes can be recorded only from inside the ventricle, and the steep slope and a short time beyond the peak occupy the interval during which signs of surface excitation are recorded. The beginning of the extrinsic part of the initial unipolar complex from all areas is simultaneous with the beginning of the more gradual slope of R.

On both the ventral and dorsal surfaces of the turtle ventricle surface excitation occurs earliest in the left-basal area and progressively later in areas toward the right of the apex. The interval between earliest and latest surface points may vary from 25 to 70 msec., varying with the season.

From the dog's ventricles the onset of contiguous spikes was found to coincide with the upstroke in the unipolar record, as from turtle hearts, but the records are sharper. Small pre-R deflections ahead of the contiguous electrode spikes are described, and they were found to be associated with the spike from the internal surface in an inconstant manner. The pre-R deflection is ascribed to conduction in fibers below the surface.

The sequence of surface excitation in the dog's ventricles was found to

confirm generally the findings of Lewis and Rothschild and C. J. Wiggers, but the maximal intervals between areas was less than many of theirs. Factors which might account for this are discussed. Comparative measurements from other investigators are considered. Leads from the internal surface of the dog's right ventricle indicate that the whole endocardial surface is activated within a very brief interval and the excitation passes through the wall much more slowly.

The spread of excitation over cats' ventricles was found to follow the same general pattern as in the dog, but the intervals are only about two-thirds as great or less. The difference may be accounted for by relative sizes.

The pattern of excitation of the ventricles of Macacus rhesus monkeys differs somewhat from that of dogs and cats, but the greatest intervals between surface points are approximately equal in cat and monkey hearts of similar sizes.

Recent fundamental experiments on the process of excitation are mentioned, and from them is suggested the possibility that the onset of the sharp contiguous electrode spike approximates the instant when the large fall of electrical impedance, and hence perhaps depolarization, occurs.

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THE REMOVAL OF DIODRAST FROM BLOOD BY THE DOG'S EXPLANTED KIDNEY

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The clearance of diodrast from plasma in human beings is believed to be approximately equal to the rate of flow of blood plasma through excretory renal tissues (Smith, Goldring and Chasis, 1938). Indirect evidence suggests that the removal of diodrast from renal blood in human beings is nearly complete (Smith, 1940).

The present report is concerned with direct measurements of the removal of diodrast from blood by the subcutaneously explanted kidney of dogs.

METHODS. One or both kidneys were subcutaneously explanted by the method of Page and Corcoran (1940), the non-explanted kidney being removed at a subsequent operation. Observations on diodrast excretion were not begun until three or more months from the time of last operation.

Diodrast, phenol red and inulin in 0.9 per cent NaCl solution were infused intravenously at about 1 cc. per minute, in concentrations designed to maintain phenol red (P) and inulin (I) at plasma levels of 0.7 and 70 mgm. per 100 cc., respectively, while the diodrast content was varied according to the plasma concentration of diodrast desired in the experiment. Administration of the infusion was preceded by a smaller priming dose of a more concentrated solution. Observations were usually begun 20 to 30 minutes after the priming dose had been given. The preparation of solutions, collection of urine and blood, determinations of phenol red and inulin concentration, calculation of renal clearances, extraction ratios and renal plasma flow have been described by Corcoran and Page (1939).

For brevity the following abbreviations are used: D, diodrast iodine concentration; PW, plasma water; CW, cell water; C_D, diodrast clearance in cubic centimeters of plasma per square meter body surface per minute; E, renal extraction ratio, which is calculated as (A-V)/A, where A and V are, respectively, the simultaneous arterial (or jugular) plasma concentration, and V is the renal venous plasma concentration, of phenol red, inulin or diodrast. Renal plasma flow (RPF) in cubic centimeters per square meter per minute was calculated and averaged from the plasma clearances

and extraction ratios of phenol red and inulin by the formula C/E. Plasma water content (P_W) was assumed to be 93 per cent and red blood cell water content (C_W) 74 per cent.

The diodrast clearance in human beings is depressed at high plasma concentrations because of overloading of the tubular excretory process, which has a limiting maximal rate (Smith *et al.*, 1938). A similar limitation in tubular excretion is present in the dog, as has been demonstrated for phenol red by Shannon (1935) and for diodrast and other substances by Smith (unpublished observations). Since increasing either RPF or D increases the load of diodrast carried to the tubules, it is appropriate, in comparing various observations, to consider this "load" (RPF $\times$ D) rather than plasma concentration alone.

Diodrast analysis. Samples of urine and plasma collected in Indianapolis were sealed in glass ampoules and mailed to New York for the determination of diodrast content by the method described by Smith, Goldring and Chasis (1938). The adequacy of this method for recovery of diodrast iodine from plasma at concentrations lower than those met in the analyses of renal venous blood was demonstrated by a substantial series of recoveries. Inorganic iodine blanks (0.02 to 0.04 mgm. per 100 cc.) were in some instances observed in the analysis of samples of plasma obtained before diodrast was given, and in these instances a correction was made by subtracting the bland from both arterial and renal venous diodrast concentration. This correction was made on the assumption that this iodine is excreted with low and negligible clearance. The method of White and Rolf (1940) was used in analyses made in most of the specimens from dogs with bilaterally explanted kidneys. Analyses of red blood cell D were obtained from the difference between whole blood and plasma values.

RESULTS. *Distribution of diodrast in plasma and red cells.* The ratio D_{CW}/D_{PW} in arterial (or jugular venous) plasma of dogs averages 0.49 and ranges from 0.33 to 0.61 in 15 observations made during constant or very slowly changing D. Ratios ranging from 0.12 to 0.67 were encountered in observations made during rapid shifts of D. Low ratios are associated with rising D and *vice versa*, and the ratio is restored towards the mean as D is maintained for 10 to 20 minutes. The value of the ratio is independent of the absolute value of D.

The ratio D_{CW}/D_{PW} in renal venous blood varies from 0.31 to 2.33 and is highest at high arterial D.

The ratio $\dfrac{D_{CW}\text{ renal vein}}{D_{CW}\text{ renal artery}}$ averages 0.72 in 11 observations at renal venous D_{PW} of 0.15 to 1.4 mgm. per cent, and varies from 0.55 to 1.28. In 6 observations at higher renal venous D_{PW} (2.0 to 3.3 mgm. per 100 cc.) the mean is 0.79.

Extraction ratio of diodrast. Plasma. The mean plasma extraction ratio of diodrast (E_p) from plasma in uninephrectomized dogs with single ex-

planted kidneys is 0.84, with extremes of 0.79 to 0.96 in 19 observations at renal loads of from 0.5 to 4.9 mgm. per square meter per minute. E_p decreases at higher plasma loads (fig. 1), no doubt because of encroachment on the maximal rate of tubular excretion. In dogs with bilaterally explanted kidneys, E_p averages 0.83 (9 observations) and ranged from 0.73 to 0.89 at renal loads of from 0.5 to 10 mgm. of iodine per square meter per minute. A few anomalously low values, possibly due to contamination of the renal venous sample with arterial blood or with urine, or to the opening of arterio-venous shunts in the kidney, are not included in this report.

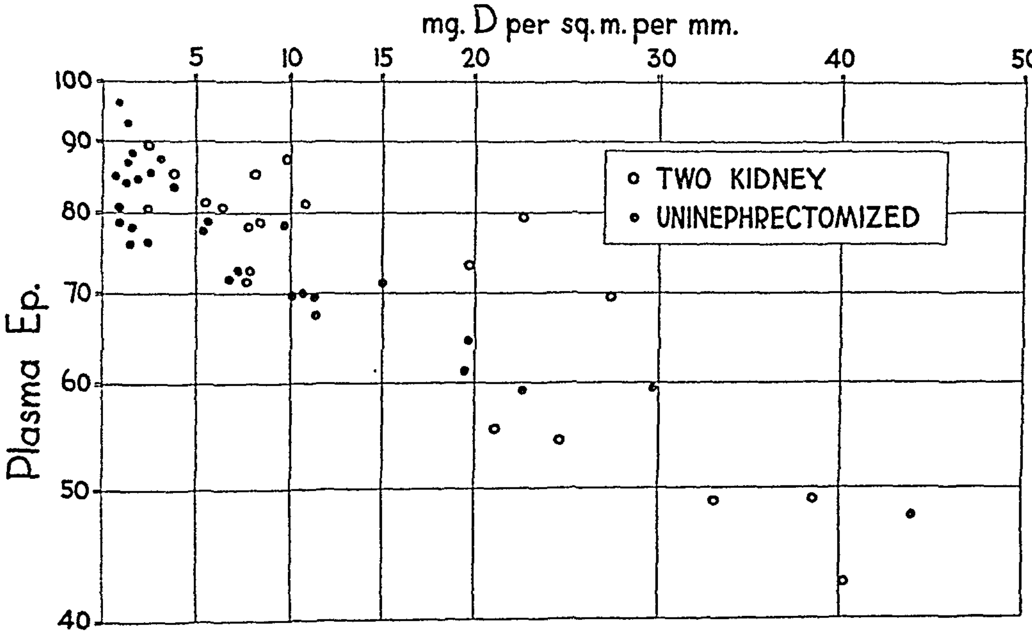

Fig. 1. The relation of renal diodrast extraction percentage to apparent renal plasma load. Ordinate: renal plasma load of D. in milligram per square meter of body surface per minute (RPF$\times$D$_p$). Abscissa: diodrast extraction percentage $\frac{(A-V)}{A}100$.

Red blood cells. Diodrast extraction from red cells (E_c) in dogs with bilaterally explanted kidneys averages 0.203 at renal plasma loads of from from 0.5 to 9.9 mgm. of iodine per square meter per minute and ranges from 0.043 to 0.514 (10 observations). At renal loads of from 10 to 40 mgm. of iodine per square meter per minute, the mean of 7 observations is 0.215. Three instances of apparent addition of diodrast to red cells in the kidney were not included in the calculation of those means.

Whole blood. Diodrast extraction from whole blood (E_b) at renal loads of from 0.5 to 10 mgm. D per square meter per minute averages 0.707 in 9 observations in a dog with bilaterally explanted kidneys, and ranged from 0.64 to 0.82.

Diodrast clearance and renal blood flow. In those experiments at low

loads (0.5 to 5 mgm. of iodine per square meter per minute) in uninephrectomized dogs, the mean ratio of plasma C_D to RPF is 0.87 (25 observations), the apparent renal plasma flow, as calculated from C_D/E_p is 1.06 RPF. Two experiments (7 observations) in dogs with bilaterally explanted kidneys yield similar values. The ratio $\frac{\text{whole blood } C_D/E_b}{\text{renal blood flow}}$ was 0.97 in these.

Discussion. *Distribution of diodrast in cells and plasma.* White (1940) has confirmed the observations of Smith, Goldring and Chasis (1938) that diodrast enters red blood cells *in vitro* very slowly, but has found that penetration *in vivo* occurs rapidly. Apparently equilibrium is reached before 20 minutes. The distribution ratio (D_{CW}/D_{PW}) found by White (0.58) is in fair agreement with our average of 0.49. The reason why diodrast is not distributed uniformly per unit of plasma and cell water is unknown.

The variability of the ratio (D_{CW}/D_{PW}) in renal venous blood and its general proportionality to the diodrast content of arterial blood is presumably due to failure of establishment of cell/plasma equilibrium in the short time of a single renal passage (White, 1940).

The diodrast content of renal venous cell water is nearly always lower than that of arterial cells. The mean ratio of these values of 0.72 found by us at low plasma levels agrees with the value of 0.77 found by White (1940).

Renal extraction of diodrast. The mean extraction of diodrast from plasma in dogs at low renal loads is apparently unaffected by uninephrectomy, although the lower tubular mass of uninephrectomized dogs causes depression of extraction at lower renal loads than in dogs in which both kidneys are present. Our mean value (0.84) is somewhat higher than that found by White (1940) in dogs with single explanted kidneys (0.74). This difference is possibly due to the fact in White's observations the mean included all data obtained at plasma D of less than 13 mgm. per 100 cc., whereas our average is based on data obtained at relatively low loads.

The mean extraction from red blood cells in our observations agrees with the value of 0.23 found by White (1940). Apparent addition of diodrast from plasma to the red blood cells during renal passage was noted in three instances. The significance of this observation is not clear.

The extraction from whole blood in our observations in dogs with bilaterally explanted kidneys is 0.707 in contrast to the value of 0.56 found by White (1940). By our average E_p of 0.84, mean E_c of 0.20 and arterial (D_{CW}/D_{PW}) of 0.48, the calculated E_b should be 0.69. Similarly, in the data reported by White (1940), the mean E_p of 0.74, E_c of 0.23 and arterial (D_{CW}/D_{PW}) of 0.57 give a calculated E_b of 0.59, as compared with the observed value of 0.57. Thus the observed E_b in both cases agree with the values calculated from independent data. This agreement suggests that the differences in E_b between White's observations and those reported here are systematic.

Diodrast clearance and renal blood flow. The contribution of a fraction of the red blood cell diodrast to the urinary excretion renders the measurement of true renal plasma flow uncertain in the absence of analysis of renal venous blood, as White (1940) has pointed out. In the present observations RPF = 0.943 C_D/E_p, as compared with 0.89 C_D/E_p in White and Heinbecker's observations (1940), deviation from unity representing the contribution of diodrast from the cells. Balancing this contribution from the red cells (6 per cent) is the incompleteness of plasma E_p (14 per cent). Diodrast plasma clearance is therefore less than true renal plasma flow and the ratio RPF/CD averages 0.87. True renal plasma flow is therefore 1.15 CD while the value obtained by White and Heinbecker (1940) was 1.2 CD.

We may add the cautionary note that because of occasional low extraction ratios observed by White and Heinbecker and ourselves, and because the explanted kidney is perhaps subject to abnormal stresses, especially on the renal vein, and frequently shows some thickening by the capsule and scarring, we are not fully convinced that the values for the extraction ratio here reported represent the normal average value in the kidney *in situ*.

SUMMARY

Diodrast intravenously infused in dogs is unequally distributed between the plasma and red blood cells, the distribution ratio between cell and plasma water being approximately 0.50.

At low renal loads, the extraction of diodrast from arterial plasma during its passage through the explanted kidneys of dogs averaged 0.84. Diodrast extraction was not affected by uninephrectomy.

Renal extraction of diodrast from arterial red blood cells averaged 0.20.

The removal of diodrast from arterial blood by the dog's explanted kidney is such that at low renal loads the ratio $\frac{\text{plasma diodrast clearance}}{\text{renal plasma flow}}$ is approximately 0.87.

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THE TRANSFER OF RADIOACTIVE SODIUM ACROSS THE PLACENTA OF THE GOAT

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This study is the fourth of a series on the comparative physiology of placental transfer. It follows observations (1, 2, 3) on placental transfer of radioactive sodium across the hemochorial placenta (maternal and fetal circulations separated by chorionic epithelium and endothelium of fetal blood vessels) and the endotheliochorial placenta (maternal and fetal circulations separated by endothelium of maternal blood vessels, chorionic epithelium and mesenchyme, and endothelium of fetal blood vessels). The placenta of the goat is syndesmochorial, i.e., the maternal and fetal circulations are separated in order by endothelium of maternal blood vessels, maternal connective tissue, chorionic epithelium, fetal connective tissue and endothelium of fetal blood vessels. As in the earlier reports, this study has as its purposes an evaluation of transfer rate across a unit weight of placenta at various stages of pregnancy; a correlation of the transfer rate and morphology of the placenta; and a comparison of the relative growth rate of the fetus and the rate at which sodium is supplied to a unit weight of fetus at different periods of gestation.

Experimental procedure. The general experimental procedure has previously been fully presented (1, 2). Radioactive sodium (Na^{24}), present as the chloride, was prepared by use of the Harvard cyclotron. Approximately 30 microcuries were taken for injection into an ear vein of a pregnant animal. The radioactivities of fetal ash and maternal plasma were measured with a pressure ionization chamber-string electrometer circuit (1). Ether was given to the animals before delivery of the fetuses by Caesarean section. Pregnant animals were obtained from a local herd of domestic goats and, except where noted, the gestation age was known.

The units of radioactivity which have been used are those previously given (1). The term "corrected" placed after values for Na^{24} means that these values have been corrected to a concentration of one beta-particle per second per cubic centimeter of maternal plasma.

Results. With the guinea pig (1), cat (2) and rat (3), the first experimental step was to establish the curve describing equilibration of the fetus

with the Na^{24} in the maternal plasma. This permitted the removal of the fetus at a time when its concentration of Na^{24}, derived from the maternal plasma, was increasing in a linear manner. In view of information gained from previous experience and the limited amount of experimental material, it was considered inadvisable to establish the equilibrium curve for the goat. Instead, the volume of distribution of Na^{24} in the goat fetus near term was assumed to be like that of the guinea pig. This value in the goat fetus at this period is probably about 30 per cent of the body weight. The transfer rate of Na^{24} is of such magnitude (fig. 4) that at the end of two hours the fetus has approximately one-sixth the concentration of Na^{24} anticipated at equilibrium. This deduction was verified in one young fetus by making sodium analyses on the maternal plasma and fetal ash, from these analyses calculating the apparent volume of distribution of Na per unit body weight, and then comparing the rate of transfer of Na^{24} to the equilibrium value established in this way. Previous experience has shown that the apparent rate of exchange between maternal plasma and fetus is linear up to and somewhat beyond one-sixth of the concentration found at equilibrium in the fetus. The routine procedure for measuring placental transfer in the goat has consequently been to remove the fetuses by Caesarean section at a known interval of about two hours after intravenous injection of Na^{24} into the mother.

The change of placental weight with change of fetal weight is given in figure 1. The lack of correlation between these two quantities is notable. The placental weight as noted refers to the combined weights of the fetal cotyledons and does not include the intercotyledonary chorion. The total Na^{24} transferred to the fetus per unit time, as this varies with change of fetal weight or gestation age, is shown in figure 2. The data of figures 3 and 4 have been derived from those of figures 1 and 2.

Figure 3 shows the changes in rate of transfer across a unit weight of placenta from about the tenth week of pregnancy until term. There is a three or fourfold increase from about the tenth to the nineteenth and twentieth weeks of gestation and then a decrease to term.

Figure 4 gives in per cent the daily relative weight increase of the fetal goat as this varies with fetal weight. The daily per cent weight increase has been calculated from that part of the data of figure 2 which relates fetal weight to known gestation age; the method of calculation has previously been given (1). Figure 4 also gives the transfer rate of Na^{24} per gram fetus per hour as this changes with fetal weight. The two curves are similar. The ratio of their ordinates at corresponding fetal weights is as follows: at 100 grams, 1.5; 200 grams, 1.4; 500 grams, 1.9; 1000 grams, 1.6; 2000 grams, 1.5.

The essential data are lacking for calculation of the safety factor for sodium (ratio of rate of supply of sodium to the fetus to rate of accretion

of sodium by the fetus) at all stages of gestation. If it be estimated, however, that the volume available to Na is 30 per cent of the body weight at

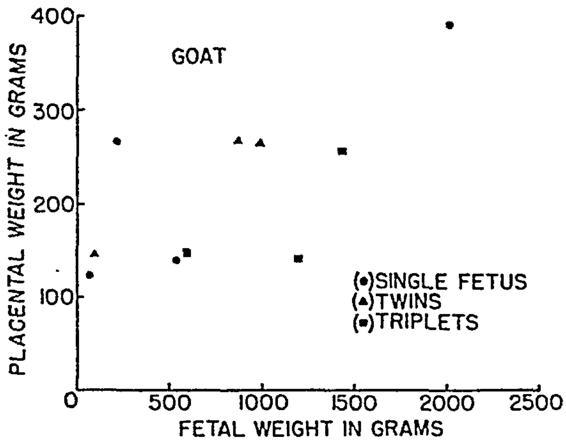

Fig. 1

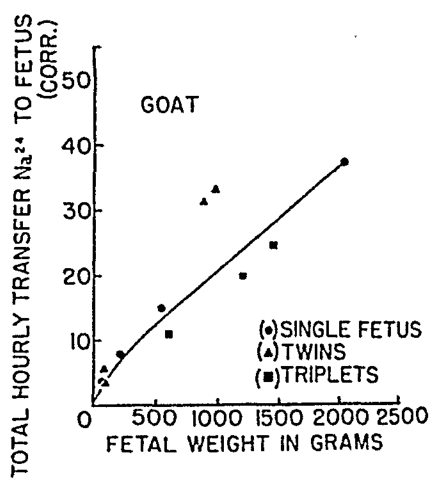

Fig. 2

Fig. 1. Variation of placental weight with variation of fetal weight. The gestation ages of the fetuses are given in figure 3.

Fig. 2. Variation of total hourly transfer of radiosodium to fetus with variation of fetal weight. The measured radioactivity of the fetal ash has in each instance been corrected to a concentration of one beta-particle per second per cubic centimeter of maternal plasma.

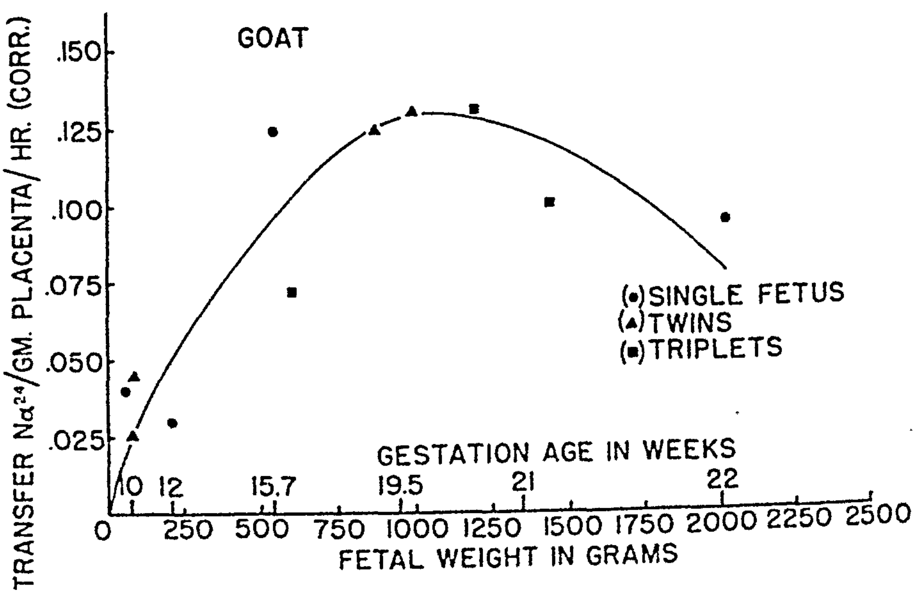

Fig. 3. Variation of transfer rate of Na^{24} per unit weight of placenta with variation of fetal weight or gestation age. The gestation age of the triplets and of the twins weighing 870 and 900 grams was unknown.

or near term, the safety factor can be calculated for this period of gestation using the formula previously derived (1): safety factor = transfer of Na^{24} per gram fetus per hour (corrected) $\times$ 24 hrs. $\times$ 100 $\div$ equilibrium con-

centration Na^{24} (corrected) × daily per cent weight increase. This calculation gives a safety factor of 100 at or near term. An analysis of the Na of maternal plasma and fetal ash of a fetus of about the same weight as the smallest shown in figure 4 gave a value for the ratio of concentration of Na in the fetus to that in the maternal plasma of 0.5. Using this value, the safety factor for the fetus of 70 grams is 28.

Discussion. The few observations (4) which have been made on the morphology of the placenta of the goat indicate that it is like that of the sheep and the conclusions drawn from the more numerous studies on the sheep have consequently been assumed to hold for the goat. The best evidence (4, 5, 6) leads to the view that within the cotyledonary placenta

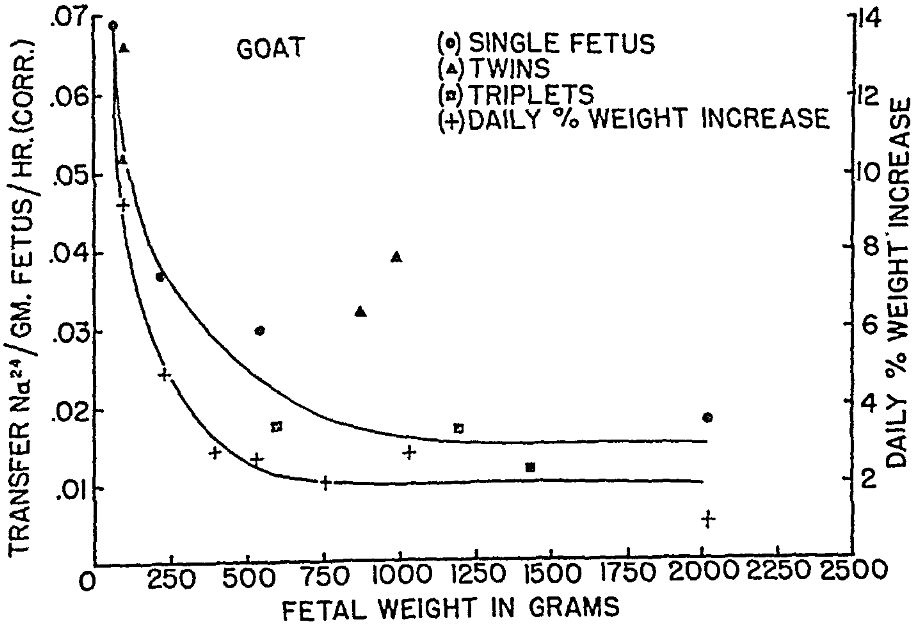

Fig. 4. Comparison of curve describing variation of daily per cent weight increase with variation of fetal weight and curve describing variation of transfer rate per unit weight of fetus with variation of fetal weight.

of these two animals, the maternal and fetal circulations are separated by endothelium of maternal blood vessels, maternal connective tissue, chorionic epithelium and mesenchyme, and endothelium of fetal blood vessels. In Grosser's classification (7), these are the characteristics of the group of placentae designated as syndesmochorial.

During the last half or third of pregnancy, several changes have been described within the cotyledonary areas. According to Assheton (5), the chorionic epithelium in the sheep consists of two layers, the outer of which is syncytial. The syncytial layer apparently does not completely disappear but becomes discontinuous in late pregnancy. Andresen (4) has also noted a thinning of the chorionic epithelium. In addition, as gestation proceeds, the fetal villi branch repeatedly and this leads to a reduction

of the connective tissue lying between their epithelium and the endothelium of maternal blood vessels. These morphological changes are reflected in the experimental data which show a three or fourfold increase in transfer rate from the ninth or tenth week to about the nineteenth and twentieth weeks of pregnancy.

As in the cases of the guinea pig, cat and rat, the curve describing the rate at which Na^{24} is supplied to a unit weight of the fetus of the goat is similar to the curve describing the relative growth rate of the goat fetus at various parts of gestation (fig. 4). The comparison of these two curves gives a measure of the adaptation of the placenta to fetal needs, for when a unit weight of fetus is reproducing itself relatively rapidly there is a correspondingly large demand for the transfer of substances across the placenta to each unit weight of fetus. It has been tentatively proposed (1, 2, 3) that a fundamental principle underlying change in placental trans-

TABLE 1

Comparison of placentae of cat and goat; and relationship between transfer rates of Na^{24} per gram fetus and relative growth rates of cat, goat and guinea pig

Values are those at the mid-point of the indicated period of gestation. Values for guinea pig and cat are taken from previously published data (1, 2).

	PERIOD OF GESTATION IN TENTHS OF TOTAL:			
	0.6–0.7	0.7–0.8	0.8–0.9	0.9–1.0
Ratio transfer rates/gm. placenta, cat to goat	0.95	1.5	1.8	1.7
Ratio relative growth rates, cat to goat	5.1	4.6	3.8	3.0
Ratio transfer rates/gm. fetus, cat to goat	2.6	2.4	2.4	2.7
Ratio relative growth rates, guinea pig to goat	4.1	4.6	4.0	4.0
Ratio transfer rates/gm. fetus, guinea pig to goat	5.2	5.7	5.9	5.7

fer during the gestation period is that the rate of placental transfer to a unit weight of fetus shall vary as does the relative growth rate of the fetus. The degree to which this hypothesis is satisfied by the data on the goat has been shown above by a comparison of the ordinates of the relative growth curve and the curve of transfer rate of Na^{24} per gram of fetus.

Not only is the relative growth rate of the fetus of a particular animal to be related to the rate of supply of a substance to a unit weight of fetus but the same sort of relationship between fetuses of different animals may be demonstrated. In table 1 the ratios of the relative growth rates of the fetuses of guinea pig and goat are given at different parts of gestation. Values are also given for the ratios of the transfer rates per gram of fetus. These two sets of ratios are remarkably alike. The values for the ratios of the relative growth rates of fetuses of cat and goat deviate more from the ratios of rates of transfer of Na^{24} per gram of fetus (table 1).

Table 1 also gives a comparison of the rates of transfer across unit weights of the placentae of the cat (2) and goat. The transfer rates per unit weight of placenta are alike in the sixth tenth of pregnancy and, indeed, the transfer rate of the cat's placenta at no time appears to be more than double that of the goat's. This is a difference not exceeding that found between placentae belonging to the same morphological group (3). From the viewpoint of rate of placental transfer as judged by Na^{24}, therefore, it appears likely that the syndesmochorial placenta of the goat may be placed in the same group as the endotheliochorial placenta of the cat. All other structures placed between the fetal and maternal circulations in the two placentae being considered more or less alike, it is not surprising that the additional maternal connective tissue of the cotyledons of the goat should have little measurable effect upon the transfer rate. Caution must be used in applying these results on the goat to the syndesmochorial placentae of other ruminants as considerable variation has been noted in the structure of the placentae of this group.

In the analysis of the observations, it will be noted that no account has been taken of the intercotyledonary placenta. This is epitheliochorial (5) and there is reason to believe that it affects in no important way the deductions drawn from the data.

These studies would have been impossible without the coöperation of Dr. Baldwin R. Curtis of the Department of Physics, Harvard University. We are grateful to him and we are indebted to the facilities offered by the Harvard cyclotron for the samples of radiosodium.

SUMMARY

1. The rates of placental transfer per unit weight of placenta have been measured with Na^{24} from a gestation age of about nine weeks until term. There is a three or fourfold increase in transfer rate from the ninth week to about the nineteenth and twentieth weeks of pregnancy.

2. The relative growth curve of the goat fetus is similar to the curve of rate of transfer of Na^{24} to a unit weight of fetus at different periods of pregnancy.

3. The placenta of the goat belongs to the syndesmochorial group. Its rate of transfer of Na^{24} per gram of placenta is of the same order of magnitude as that of the endotheliochorial placenta of the cat at comparable stages of pregnancy.

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THE TRANSFER OF RADIOACTIVE SODIUM ACROSS THE PLACENTA OF THE RABBIT

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In Grosser's classification (1) the placenta of the rabbit belongs to the hemochorial group. It is included in this series of investigations on placental transfer because the rabbit is widely used in studies on fetal physiology; and because, by comparison with the results obtained from the hemochorial placentae of the guinea pig (2) and rat (3), it will yield further evidence of the degree of variation in placental transfer among members of the same morphological group.

Experimental procedure. The general experimental procedure has previously been fully presented (2, 4). Radioactive sodium (Na^{24}), present as the chloride, was prepared by use of the Harvard cyclotron. Approximately 2 microcuries were taken for injection into an ear vein of a pregnant animal in all but the earliest stages of pregnancy when as much as 10 microcuries were used. The radioactivities of fetal ash and maternal plasma were measured with the pressure ionization chamber-string electrometer (2). Ether was given just before delivery of the fetuses by Caesarean section.

The units of radioactivity which have been used are those previously given (2, 3). The term "corrected" placed after values for Na^{24} means that these values have been corrected to a concentration of one beta-particle per second per cubic centimeter maternal plasma.

Measurements were made on 63 fetuses obtained from 17 pregnant animals. Twelve of these animals were of homogeneous breed and of known gestation age.

Results. *Establishment of equilibrium between maternal plasma and fetus.* In the cases of the guinea pig (2), cat (4) and rat (3) the first experimental step was to establish the curve describing equilibration of the fetus with Na^{24} in the maternal plasma. This permitted the removal of the fetus at a time when its concentration of Na^{24}, derived from the maternal plasma, was increasing in a linear manner. In view of our previous experience, it was considered unnecessary to establish the equilibrium curve for the rabbit. Instead, the fetal concentration of Na^{24} at equilibrium was

obtained for several individuals and also measurement was made of the amount of Na^{24} which had been transferred to the fetus at the end of half an hour. At the end of half an hour, the fetus had attained a concentration of Na^{24} equal to one-fifth to one-sixth of the equilibrium value. This finding is like that in the rat. The routine procedure for measuring placental transfer in the rabbit was consequently the same as in the rat. Fetuses were removed at an exactly known interval, about half an hour, after intravenous injection of Na^{24} into the mother.

Rates of placental transfer. The change of placental weight with change of fetal weight is given in figure 1. The total Na^{24} transferred to the fetus per unit time as this varies with change of fetal weight is shown in figure 2. From these data have come the curves of figures 3 and 4.

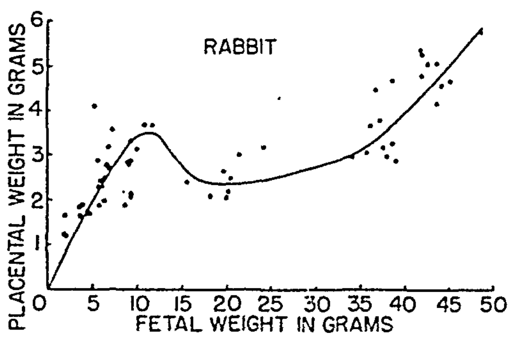

Fig. 1

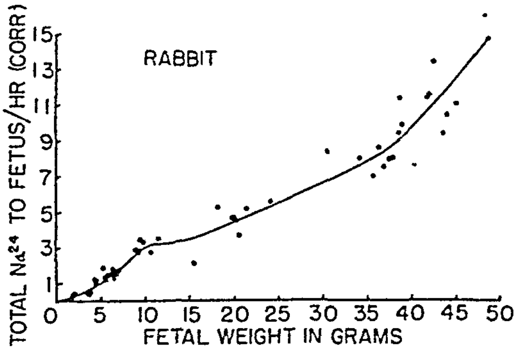

Fig. 2

Fig. 1. Variation of placental weight with variation of fetal weight. The gestation ages of the fetuses are given in figure 3.

Fig. 2. Variation of total hourly transfer of radiosodium to fetus with variation of fetal weight. The measured radioactivity of the fetal ash has in each instance been corrected to a concentration of one beta-particle per second per cubic centimeter of maternal plasma.

Figure 3 shows the changes in rate of transfer across a unit weight of placenta from the 18th day of gestation until term. At the 18th day the transfer rate is 0.25 unit and at the 30th day, 2.75 units. During these 12 days of pregnancy, consequently, the transfer rate per unit weight of placenta increases 11 times.

Figure 4 gives the rate at which Na^{24} is supplied from the maternal plasma across the placenta to each gram of fetus as this rate varies with fetal weight. This rate rises from a low value in the fetus of 4 grams to a peak which occurs at a fetal weight of about 8 grams (gestation age 22 days); then falls until term is approached when there is apparently a second rise. Figure 4 also gives the curve of the daily per cent weight increase. This has been calculated as previously explained (2) from our data on animals of known gestation age except for the first point corresponding to a fetal weight of 0.5 gram which has been taken from the data of Hammond

(5). The curve of relative weight increase as calculated from Hammond's data agrees closely with the curve derived from our data. The curve of

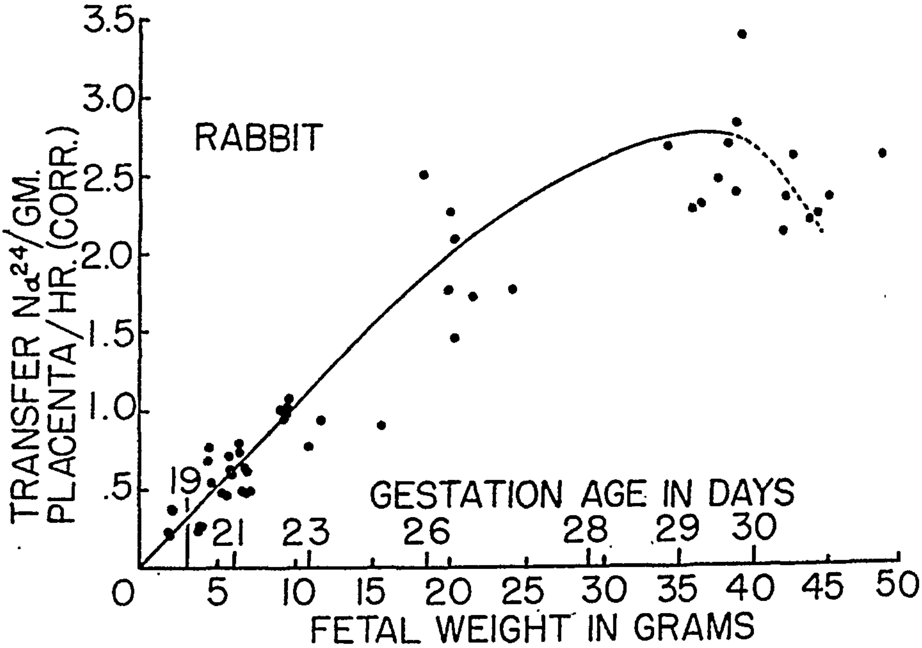

Fig. 3. Variation of transfer rate of Na^{24} per unit weight of placenta with variation of fetal weight or gestation age.

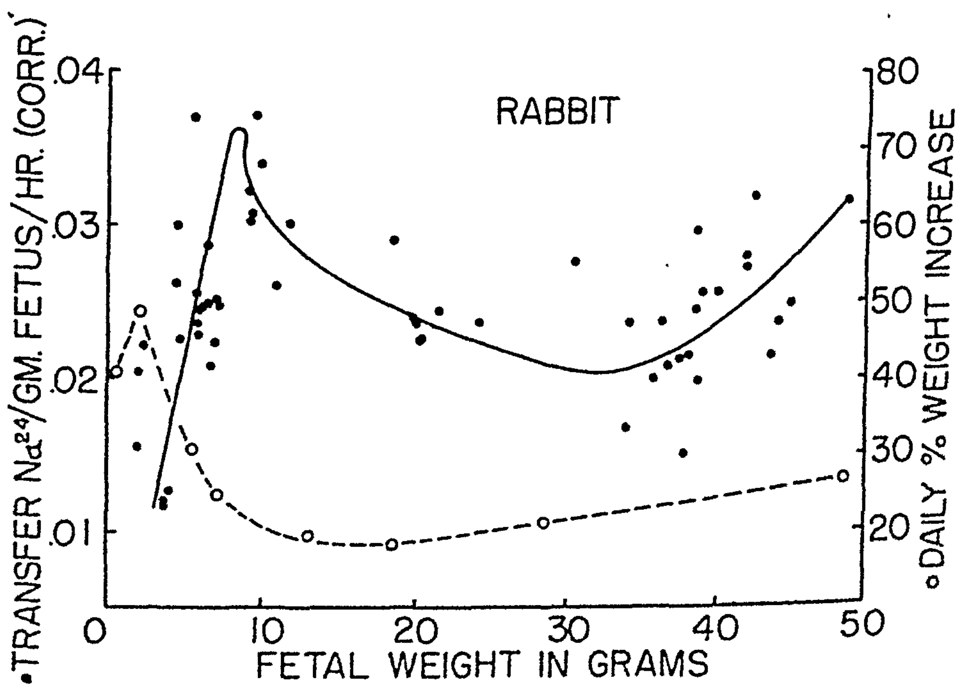

Fig. 4. Comparison of curve describing variation of daily per cent weight increase with variation of fetal weight and curve describing variation of transfer rate per unit weight of fetus with variation of fetal weight.

daily per cent weight increase has been found to be similar to the curve of rate of transfer of Na^{24} per gram of fetus in the case of other animals. In the case of the rabbit, however, the maximum in the curve of the daily per cent weight increase occurs at a fetal weight of 2 grams (gestation age 18 days) or about four days before the maximum is reached in the transfer rate of Na^{24} per gram of fetus. There is also a lag of approximately two days in the terminal rise in transfer rate per gram of fetus as compared to the terminal rise in the relative growth rate. Due to this difference in the time of occurrence of their maxima, the two curves are dissimilar up to a fetal weight of about 20 grams (gestation age 26 days). At greater fetal weights the correspondence between the two curves is more exact, the maximum variation in the ratios of their ordinates being from 2.7 to 4.

Fetal need for Na relative to supply across placenta. By using the transfer rate of Na^{24} per gram of fetus, the fetal equilibrium concentration of Na^{24} and the daily per cent growth rate of the fetus in an equation previously given (2, 3), the safety factor (ratio of the rate of supply of Na to

TABLE 1

Data necessary for calculation of safety factor

FETAL WEIGHT	TRANSFER Na^{24} PER GRAM FETUS PER HOUR (CORRECTED)	DAILY PER CENT WEIGHT INCREASE	EQUILIBRIUM CONCENTRATION; Na^{24} PER GRAM FETUS (CORRECTED)	SAFETY FACTOR
grams				
10	0.33	22	0.63	57
40	0.23	25	0.57	39

the fetus to the rate of accretion of Na by the fetus) for Na can be calculated. The values for the safety factor for the two fetal weights at which the equilibrium concentration of Na^{24} was determined are given in table 1.

Discussion. The transfer rate of Na^{24} per unit weight of placenta increases in the rabbit about 11 times from about the eighteenth to the thirtieth day of pregnancy. Results showing an increase in rate of placental transmission with increase in gestation age have also been reported by Lell, Liber and Snyder (6) who followed the urinary excretion of phenolsulphonephthalein by the mother after injection of the substance into the fetus; and by Rodolfo (7) who studied the transfer of antibodies across the placenta.

A considerable part of this increase in transfer rate is likely due to a true increase in placental permeability caused by changes in the tissues separating the maternal and fetal circulations. These changes as they occur on particular days of gestation have been studied by Mossman (8). At about the nineteenth day, the two circulations are separated by endothelium of fetal blood vessels and chorionic epithelium, i.e., the placenta is truly

hemochorial. Later stages of the placenta show progressive thinning of that chorionic epithelium and its progressive disappearance in many areas at which, consequently, the placenta is hemoendothelial. The evidence is clear that the barrier between the two circulations diminishes with advance of pregnancy and this change is to be correlated with the increase in transfer rate of Na^{24} per unit weight of placenta.

Circulatory changes which may affect the rate of placental transfer in the rabbit are not completely understood. Measurement of the rate of blood flow through the vessels of the pregnant uterus of the rabbit during the last half of pregnancy, however, gives evidence which suggests that during this period the rate of blood flow through the decidua basalis about doubles (9).

TABLE 2

Comparison of placentae of rabbit and guinea pig; and relationship between relative growth rates and transfer rates of Na^{24} per gram fetus of rabbit and guinea pig

Values are those at the mid-point of the indicated period of gestation. Values for guinea pig are taken from previously published data (2). The values on transfer rates in the rabbit have been corrected for the half-hour delivery time as previously explained (3).

	PERIOD OF GESTATION IN TENTHS OF TOTAL:		
	0.7-0.8	0.8-0.9	0.9-1.0
Transfer rate Na^{24}/gm. placenta, guinea pig	0.7	1.9	1.9
Transfer rate Na^{24}/gm. placenta, rabbit	0.6	1.8	2.1
Ratio relative growth rates, rabbit to guinea pig	2.5	2.4	3.3
Ratio transfer rates Na^{24}/gm. fetus rabbit to guinea pig	2.3	2.4	3.3

The rabbit placenta is the third member of the hemochorial group which has been studied with Na^{24}. A comparison of the transfer rate of Na^{24} per unit weight of its placenta with that of the guinea pig and rat gives a measure of the physiological variation among members of the same group. Such a comparison has been presented for the guinea pig and rat (3) and the transfer rates across unit weights of these two placentae have been found to be closely alike. The same sort of comparison is made between the rabbit and guinea pig placentae in table 2. It is apparent that the two placentae agree in their transfer rates per unit weight within narrow limits at comparable stages of pregnancy.

In all animals previously studied (2, 3, 4, 10) the curve of daily per cent weight increase has been similar to that curve of transfer rate of Na^{24} per gram of fetus. The rabbit is an exception in that the maximum in the curve of relative weight increase precedes by about four days the maximum

in the curve of transfer rate and the similarity between the two curves is not evident prior to a fetal age of 26 days. The significance of the relationship between these two curves as an index of the adaptation of the placenta to the needs of the fetus has previously been given (2, 3).

It has been pointed out that not only is there frequently a similarity in the relative growth curve and the curve of rate of transfer of Na^{24} per gram fetus during the development of a particular fetus but that the same sort of relationship may hold among fetuses of different animals (3, 10). A comparison of this latter kind is made between the fetuses of guinea pig and rabbit in table 2. At equivalent parts of the gestation period, the rabbit fetus reproduces its own weight between 2 and 3 times as rapidly as does the fetus of the guinea pig. In correspondence with its greater rate of growth, a unit weight of fetus of the rabbit receives across its placenta a supply of Na^{24} between 2 and 3 times that received by a unit weight of the fetus of the guinea pig.

It is a pleasure to express our gratitude to Dr. Baldwin R. Curtis who has generously supplied us with samples of radiosodium made with the Harvard cyclotron.

SUMMARY

1. The rates of placental transfer per unit weight of placenta have been measured in the rabbit from the eighteenth day of pregnancy until about term. There is an elevenfold increase in transfer rate per gram of placenta over this period.

2. The placenta of the rabbit belongs to the hemochorial group. The rate of transfer of Na^{24} across a unit of its weight agrees closely with that of the placentae of the guinea pig and rat which also are hemochorial.

3. The relative growth curve of the rabbit fetus is similar to the curve of rate of transfer of Na^{24} to a unit weight of fetus only after the twenty-fifth day of pregnancy. Prior to this, large changes in the relative growth curve anticipate similar changes in the curve of transfer rate per unit weight of fetus by about 4 days. Differences in the relative growth rates of guinea pig and rabbit are to be correlated with corresponding differences in the rate of transfer per unit weight of the respective fetuses.

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THE EFFECT OF BARBITAL ANESTHESIA ON TEMPERATURE REGULATION[1]

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In studying mammalian temperature regulation, the physiologist is handicapped by having to use anesthetized animals. The excitement and discomfort resulting from mild experimental procedures without anesthesia cause profound disturbances in temperature regulation. When anesthetics are used to eliminate these difficulties, the anesthetic will often introduce disturbing factors as detrimental as the excitement. Much of the past experimental work on temperature regulation has been done on animals under anesthesia and many problems now awaiting solution could best be performed in this condition. In order to evaluate and interpret such work, it is necessary to know to what extent anesthetics, which are commonly used for experimental physiology, depress temperature regulating mechanisms. Recently we have developed standardized tests for shivering and the vasomotor response in which measured stimuli have invoked a measured response in a controlled environment. These tests have been made on the same dog with and without anesthesia in order to obtain quantitative information on the depression caused by the barbital anesthetics on the response to cold.

It is well known that many anesthetics depress temperature regulatory functions. The drop of body temperature in the usual laboratory environment which follows anesthesia has been observed by many investigators but quantitative investigations on the extent, duration and functional type of depression are practically non-existent. Deuel, Chambers and Milhorat (1926) anesthetized dogs with amytal and noted a slight fall in oxygen consumption rate which, in the majority of the experiments, was less than 10 per cent of the basal value while the body temperature was reduced 2 to 3 degrees. Eddy (1929) found that diethyl barbituric acid, 42 mgm. per kilo by stomach tube, reduced the body temperature of cats two hours after anesthesia 0.1 to 0.5 degree. Ellis and Barlow (1924-5) used higher doses of barbital, 182 mgm. per kilo intraperitoneally, and found a de-

[1] Technical assistance in this investigation was furnished by Onni Overhouse and George Cordes of the Works Progress Administration, Official Project number 665-71-3-69, subproject number 295.

pression of body temperature which, during the course of anesthesia, dropped to values as low as 3 degrees below normal and returned to normal after 48 hours. Page and Coryllos (1926) found that amytal anesthesia caused a slight drop in blood pressure of dogs, and Lepper (1926) observed that small doses of veronal caused a constriction of ear vessels while larger doses produced dilatation. These few observations, taken from papers in which temperature regulation was not the primary interest of investigation, are in agreement with our observations that barbital anesthesia causes a drop in body temperature with peripheral vasodilatation and impaired shivering in the usual laboratory environment. These previous investigations, insofar as they are concerned with temperature regulation, are far from being complete. In many cases room temperature was not controlled nor were skin and rectal temperatures followed through the course of anesthesia and correlated with temperature regulatory mechanisms.

In our investigations sodium pentobarbital and amytal have been used in dosages suitable for surgical anesthesia and physiological experimentation. These anesthetics have been chosen on account of their ease and convenience of administration and their extensive use by physiologists. Control experiments have been made in which normal responses have been measured without anesthesia. Identically similar experiments were performed on the same dogs under anesthesia. The reactions which have been measured are shivering and the vasomotor response.

EXPERIMENTAL. *Part I. Depression of shivering and vasomotor control with anesthetic doses of barbitals.* Four short haired bull terrier type dogs weighing 10 to 15 kilos were carefully chosen and trained for testing without anesthesia. The animals were placed on a mechanical shivering recorder, as described by Hemingway (1940), in an air conditioned room in which the air temperature was 22.0 ± 0.5 degrees, the relative humidity 50 ± 5 per cent, and the air velocity 25–40 feet per minute. Diathermy electrodes were placed on the dog, one electrode being beneath the recumbent animal while the other was placed on the upper flank. Temperatures of the skin of the ears, foreleg, thorax, beneath the diathermy electrodes, and of the rectum were measured by thermocouples. The dog was trained to lie quietly on the recorder and as a result of resting in this cool environment, the skin and rectal temperatures fell, producing first vasoconstriction, noted by a rapid fall of ear temperature, and then shivering which prevented a further fall in temperature. When the steady state was reached the diathermy current was turned on and the dog heated at a rate equal to the b.m.r. As the heating progressed shivering stopped and finally vasodilatation occurred. Temperatures and shivering intensity were plotted against time, as shown in figure 1. Each experiment lasted 3 to 8 hours.

The same experiment was performed on the same dog on another day after being anesthetized. Sodium pentobarbital was given both intravenously-intramuscularly and intraperitoneally in doses of 35 mgm. per

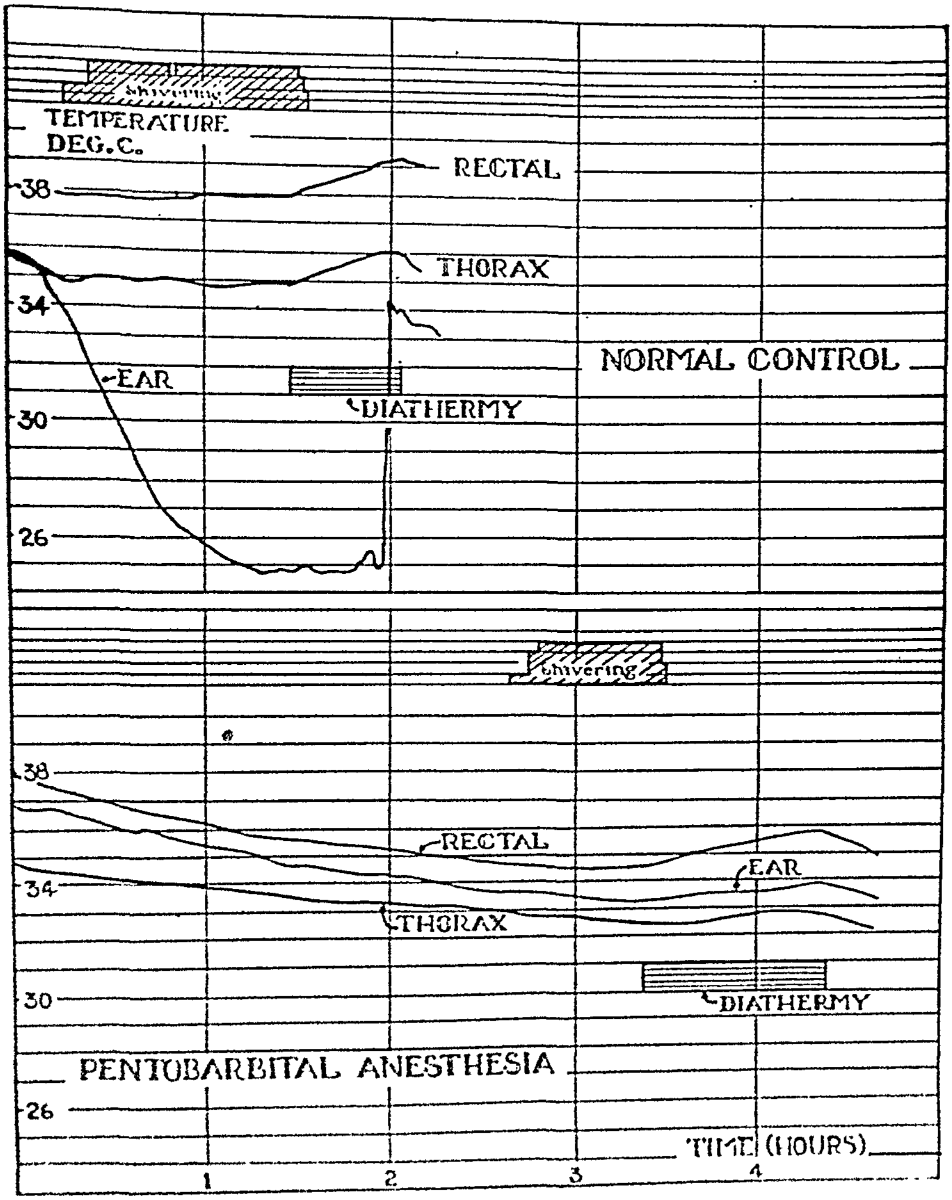

Fig. 1. The change of skin and rectal temperatures of a normal and an anesthetized dog in a cool room before and after diathermy heating. Shivering is indicated on an arbitrary scale with no shivering indicated as 0 and most intense shivering as 5.

kilo. When given intravenously two-thirds of the full dose was injected at once with the remainder being injected intramuscularly 45 minutes later. When administered intraperitoneally the full dose was injected at one time. One or two experiments were made on each mode of anesthe-

tization. The same procedure was used when sodium amytal was given except that the total dosage was 55 mgm. per kilo. On each dog at least 4 control experiments were performed.

Part II. Duration of temperature regulatory depression with anesthetic doses of barbitals. In order to investigate the extent of recovery of shivering and vasomotor control during the 3 to 4 hour period of anesthesia, a series of experiments was made in which the following procedure was adopted for one dog (B). The dog was placed on the shivering recorder in the constant temperature-constant humidity room, and the thermocouples and diathermy electrodes were attached. The diathermy current was started and regulated to maintain the body temperature constant with shivering and vasoconstriction being prevented. After 30 minutes of rest sodium pentobarbital, 35 mgm. per kilo, was injected intraperitoneally in a single dose. The diathermy current was continued and varied to keep the body temperature from falling under anesthesia. After a time interval of from 0 to 240 minutes the diathermy current was discontinued and cooling was allowed to proceed while measurements of temperature and shivering were made. In a series of experiments the heating period between injection of the anesthetic and cessation of diathermy was set at 0, 30, 60, 90, 120, 180 and 240 minutes. Thus the animal was not allowed to cool until after periods of anesthesia having these duration times. For each experiment a time-temperature graph similar to those of figure 1 was made.

Results. In figure 1 are two graphs, one showing the response of a normal dog to cold followed by diathermy heating, and the other graph the response of the same dog under pentobarbital anesthesia in an identical experiment. A normal dog responds to a cool environment by peripheral vasoconstriction, which is most marked in the ear vessels. The result is a drop in ear temperature to 25 or 27 degrees. Simultaneously, or a few minutes later, shivering occurs, after which the body temperature remains at a constant level. In a dog anesthetized with either of the two barbiturates, both the vasomotor and shivering mechanisms are partially or completely paralyzed. In the experiment recorded by the lower graph of figure 1, which indicates marked temperature regulatory impairment, peripheral vasoconstriction did not occur in spite of a drop in rectal temperture of several degrees. Shivering did occur but the temperature thresholds were depressed several degrees.

There was a considerable variation in response between different anesthetized animals and the same animal on different days. The usual temperature-time curves during anesthesia were intermediate between the two extremes represented in figure 1, i.e., the normal response and that of the deeply narcotized animal with severe temperature regulatory paralysis.

Part I. Shivering and vasomotor depression. For each dog at least 4 con-

trols and 4 anesthetic experiments were performed. A graph similar to those of figure 1 was drawn and the significant data taken from each graph is listed in tables 1 and 2. For the 4 control experiments, maximum,

TABLE 1

Shivering

DOG	ANES. METHOD	RECTAL TEMPERATURE				SKIN TEMPERATURE (THORAX)		
		Shivering		Min. temp. reached	Time to reach min. temp.	Shivering		Min. temp. reached
		Starts	Stops			Starts	Stops	
A (max.)	Con.	36.8	37.2	36.8	70	34.2	34.0	33.5
A (min.)	Con.	35.9	36.2	35.9	40	32.5	33.2	32.5
A (ave.)	Con.	36.4	36.8	36.4	55	33.5	33.7	33.1
A	NP	33.0	38.3	33.0	150	30.1	33.8	30.1
A	NV	34.0	38.7	34.0	135	30.8	33.6	30.7
A	AP	N.S.	N.S.	32.4	78	N.S.	N.S.	29.6
A	AV	33.6	38.3	33.1	85	30.1	29.7	29.4
B (max.)	Con.	38.1	38.5	37.9	100	34.8	35.5	34.6
B (min.)	Con.	37.6	38.0	37.2	10	34.3	34.3	33.5
B (ave.)	Con.	37.8	38.2	37.6	60	34.6	34.8	34.2
B	NP	34.4	36.6	34.4	105	32.3	34.4	32.3
B	NV	35.0	36.1	35.0	165	31.2	31.5	31.2
B	AP	36.4	39.8	36.1	80	33.1	36.6	33.1
B	AV	N.S.	N.S.	32.5	70	N.S.	N.S.	30.1
C (max.)	Con.	38.3	38.6	38.2	105	34.2	35.0	34.0
C (min.)	Con.	38.0	38.2	37.8	50	33.4	33.4	33.3
C (ave.)	Con.	38.1	38.4	38.0	75	33.8	34.3	33.7
C	NP	37.4	40.7	37.3	70	33.9	35.2	33.1
C	NV	36.1	36.3	36.1	50	31.1	31.1	31.1
C	AP	36.6	38.1	36.1	58	33.0	34.1	32.6
C	AV	36.1	36.7	36.1	65	32.0	31.5	31.1
D (max.)	Con.	37.9	38.3	37.9	95	35.4	35.3	35.7
D (min.)	Con.	37.7	38.1	37.4	40	33.6	34.9	33.9
D (ave.)	Con.	37.8	38.2	37.7	63	34.8	35.1	34.5
D	NP	36.0	37.9	36.0	115	32.0	32.8	31.8
D	AP	35.5	36.0	34.9	135	32.1	32.5	31.6
D	AV	35.5	37.1	35.2	80	31.0	31.7	31.0

N.S., no shivering; Con., control, no anesthesia; N, pentobarbital Na; A, amytal Na; P, intraperitoneal injection; V, intravenous-intramuscular injection.

minimum and mean values are given. The letters N and A denote sodium pentobarbital and amytal, respectively, while P and V denote intraperitoneal and intravenous-intramuscular administrations, respectively. Table 1 contains shivering data and table 2 vasomotor data. Table

1 contains the rectal and thoracic skin temperatures at which shivering started during cooling and stopped after application of diathermy. The minimum temperatures reached and the times required to reach minimum temperatures are also given. Shivering started usually within 1 to 2 hours after anesthesia with rectal temperatures of 33 to 36 degrees while

TABLE 2

Vasomotor

DOG	ANES. METHOD	VASOCONSTRICTION			VASODILATATION		
		Rectal t.	Thorax t.	Resting time	Rectal t.	Thorax t.	Heating time
A	Con. (max.)	37.1	34.5	58	38.0	34.3	92
A	Con. (min.)	36.1	32.9	31	37.2	33.2	31
A	Con. (ave.)	36.6	33.5	44	37.7	33.6	44
A	NP	33.5	30.8	42	38.8	33.8	112
A	NV	No vasoconstriction					
A	AP	32.6	30.0	68	36.5	31.4	100
A	AV	36.1	32.4	40	37.7	30.6	151
B	Con. (max.)	38.6	35.7	28	39.0	37.6	103
B	Con. (min.)	38.0	34.6	15	38.7	34.7	23
B	Con. (ave.)	38.2	35.2	22	38.9	35.5	77
B	NP	35.7	32.6	60	37.0	33.1	54
B	NV	34.9	31.3	140	38.3	33.1	240
B	AP	36.5	33.5	58	39.8	36.4	100
B	AV	37.3	33.6	27	38.5	33.9	115
C	Con. (max.)	38.5	34.6	62	39.0	35.1	38
C	Con. (min.)	38.1	33.6	27	38.7	34.8	13
C	Con. (ave.)	38.4	34.3	42	38.9	34.7	21
C	NP	37.4	35.0	45	39.7	35.1	48
C	NV	36.3	32.3	65	38.6	32.4	240
C	AP	36.3	32.9	52	38.1	34.1	25
C	AV	36.7	32.1	28	38.1	31.9	110
D	Con. (max.)	38.3	35.5	85	39.0	36.3	63
D	Con. (min.)	37.5	33.5	43	38.5	33.6	3
D	Con. (ave.)	37.9	35.1	62	38.7	35.2	25
D	NP	No vasoconstriction					
D	AP	35.2	31.9	95	36.3	32.6	32
D	AV	35.4	31.4	73	38.0	32.3	19

the normál rectal threshold temperature for shivering is 35.9 to 38.3 degrees. Dog D was unfortunately killed as a result of a dog fight before the last experiment was performed; hence the results of intravenous pentobarbital anesthesia for dog D are missing.

Table 2 contains the vasomotor data. The rectal and skin thoracic

temperature thresholds are given at which peripheral vasoconstriction occurred as a result of resting in a cool room and at which vasodilatation occurred when the animals were subsequently heated by diathermy at a heating rate equal to the b.m.r. In the column designated as "heating time" the time in minutes of diathermy is given which was required to produce vasodilatation. Since the heating rate was kept constant, this time interval is proportional to the heat dosage required to produce vasodilatation.

Part II. Duration of temperature regulatory depression. The significant data, collected from the time-temperature graphs of experiments in which the effect of duration of anesthesia on cold response depression was investigated, are given in table 3. The "anesthetic diathermy time" is the time interval between injection of the anesthetic and cessation of the diathermy current. It is the time interval of anesthesia before cooling in which the rectal temperature was prevented from falling by diathermy. The table contains both rectal and skin thoracic temperatures at which vasoconstriction and shivering occurred as well as the time interval, "cooling time", between cessation of diathermy and the onset of shivering or vasoconstriction. In the normal unanesthetized dog used for these tests, vasoconstriction occurred with a rectal temperature of 38.2 degrees and a thoracic skin temperature of 34.0 degrees after 13 minutes of cooling. Shivering occurred after 27 minutes of cooling when the rectal temperature had reached 37.9 degrees and the skin thoracic temperature 34.0 degrees.

TABLE 3

Duration of temperature regulatory depression by sodium pentobarbital

CONDITION	VASOCONSTRICTION			SHIVERING		
	Rectal temp.	Thoracic temp.	Cooling time	Rectal temp.	Thoracic temp.	Cooling time
Control—no anesthetic	38.2	34.0	13	37.9	34.0	27
Anes diathermy time = 0	36.1	31.5	40	31.4	28.1	343
	34.7	30.8	105	32.5	29.2	230
Anes diathermy time = 30	35.6	31.6	63	32.4	29.1	280
Anes diathermy time = 60	35.4	31.5	100	33.6	30.1	215
Anes diathermy time = 90	36.3	32.7	48	34.8	31.6	132
Anes diathermy time = 120	35.4	31.6	90	34.1	30.8	158
Anes diathermy time = 150	36.0	32.6	78	35.6	32.4	118
Anes diathermy time = 180	†	†	†	34.7	32.0	164
Anes diathermy time = 240	*	*	*	*	*	*

* Dog awakened when heating discontinued. Running movements and struggling prevented a fall in temperature and obscured shivering.

† Ear vessels constricted while dog was being heated.

As a result of anesthesia the cooling time is prolonged and the threshold temperatures lowered. There is only slight recovery of shivering and vasoconstriction 2 to 3 hours after anesthesia. For example, immediately after anesthesia shivering commenced at a rectal temperature of 31.4 to 32.5 degrees while 2 to 3 hours after anesthesia shivering commenced with rectal temperatures of 34.1 to 35.6. The animal awakens before there is recovery from the depression of shivering and thermal vasoconstriction.

Discussion and conclusion. For experimentation with dogs barbital anesthesia is widely preferred by physiologists. The anesthetic can be easily administered and anesthesia after a single dose lasts 2 to 4 hours. There is no appreciable effect of the barbital anesthetics on blood pressure and respiration is only slightly depressed. There is no appreciable fall of basal metabolic rate provided the body temperature is maintained. The main depressant autonomic effect is on temperature regulation. Our experiments were performed to determine how extensive this depressant action is and to what extent a physiologist is justified in using the barbitals for acute experiments on temperature regulation.

The results indicate that there is a considerable depression of both peripheral and rectal threshold temperatures at which shivering and thermal vasoconstriction commence. The depression amounts to as much as 1 to 5 degrees. Since, according to current theories, the mechanisms which protect against cold are activated when peripheral and central temperatures fall below a definite threshold value, the lowering of this threshold indicates considerable impairment by the anesthetics of the physiological temperature regulating apparatus. However, in only about ten per cent of the experiments was peripheral vasoconstriction or shivering completely inhibited. This indicates a lack of precision of control but not complete absence of temperature regulation.

There was considerable variability with the same experiment repeated on different days using the same dog. Due to this variability there was no detectable difference in the depression of temperature regulation caused by sodium amytal or pentobarbital, nor was there any difference between the intravenous and intraperitoneal methods of administering the anesthetic.

The depression of temperature regulation against cold caused by these barbiturates lasted 3 to 4 hours and exceeded the period of anesthesia. There was only a slight recovery near the end of the anesthetic period.

SUMMARY

In a cool environment of 22.0°C. and 50 per cent relative humidity, trained dogs were allowed to rest until shivering and peripheral vasoconstriction had occurred. The animals were then heated by diathermy at a rate equal to the b.m.r. until shivering was inhibited and vasodilatation

had occurred. The experiments were performed with and without amytal and pentobarbital anesthesia. Shivering was measured on a mechanical recorder and skin and rectal temperatures were measured by thermocouples. It was found that shivering and vasomotor temperature thresholds were suppressed by the anesthetics 1 to 5 degrees, and the small differential temperature range of normal animals was replaced by a differential having a 3 to 5 fold range as a result of anesthesia. Barbital anesthesia in doses required for surgical procedures suppresses the responses to cold but does not completely inhibit them. The order of suppression for temperature regulatory processes in barbital anesthesia is, in order of decreasing severity, 1, body temperature; 2, shivering; 3, vasomotor reflexes.

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AN ANALYSIS OF HYPOTHALAMIC CARDIOVASCULAR CONTROL

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The buffer reflexes, centrally integrated in the lower brainstem, serve to adjust the rate of the heart and the caliber of the vessels so that a sufficient volume flow of blood is maintained at a satisfactory pressure. But lability of control of the cardiovascular system is necessary to enable the organism to meet environmental stresses. A rise in blood pressure, an increase in heart rate, and a redistribution of blood, provide for the increased activity so commonly a part of affective behavior. A shift of blood between viscera and skin provides a means of dissipating or conserving heat. The dominant rôle assigned to the hypothalamus in emotional expression (Bard, 1929), and in thermal regulation (Ranson and Magoun, 1939) suggests that this central nervous level contributes a very necessary element of lability to medullary cardiovascular control.

Karplus and Kreidl (1928), Kabat, Magoun and Ranson (1935), Ectors, Brookens and Gerard (1938) and others have shown that electrical stimulation of the hypothalamus in the anesthetized animal leads to an increase in heart rate and a rise in blood pressure. Morrison and Rioch (1937) and Magoun (1938) demonstrated that these cardiovascular responses are independent of descending pathways from the cerebral cortex and result from activation of projection systems which take origin within the hypothalamus. The course of the descending hypothalamic connections, through the tegmentum and central gray of the mesencephalon, pons and into the medulla, brings them in close relation with those medullary centers which reflexly control the heart and blood vessels (Magoun, Ranson and Hetherington, 1938; Wang and Ranson, 1939). Through these connections it is possible that the hypothalamus might modify the basic reflex pattern laid down in the medullary centers for cardiovascular control.

One of the most effective methods of analysis of this problem is a study of the discharge of impulses initiated in sympathetic motor neurones by stimulation of the hypothalamus, and a consideration of how this activity is integrated into the reflex control of the cardiovascular system. Such a

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procedure has distinct advantages as compared with observation of the response of effector organs. Because of the latency and inertia of these organs, they cannot be used to investigate the precise nature of rapidly occurring neural processes. We have therefore recorded the activity in peripheral sympathetic nerve trunks supplying heart and blood vessels during stimulation of the hypothalamus and have correlated that activity with the response induced in the cardiovascular system. Even the discharge of impulses in a peripheral nerve trunk is not entirely suitable and we have accordingly carried out certain portions of our investigation on single units.

METHODS. Experiments were performed on cats anesthetized with 20 to 30 mgm. per kgm. of nembutal, or 50 mgm. per kgm. of chloralosane, given intravenously. Bipolar needle electrodes were introduced into the hypothalamus by means of the Horsley-Clarke stereotaxic instrument, and brief repetitive condenser discharges delivered at controlled intensities and frequencies. At the end of each experiment small electrolytic lesions were placed to mark the position of the electrode tips in the brain. The animal was perfused with formalin, the brain removed, and after a period of hardening, the brain was sectioned grossly to determine the site of stimulation. In representative experiments, the brains were embedded in nitrocellulose, sectioned at 50 microns, stained by the Weil method and examined to determine the exact placement of the electrodes.

Two nerves were used for recording sympathetic neural activity, the cervical sympathetic and the inferior cardiac nerve. The inferior cardiac nerve was approached by removal of the chest wall and careful dissection of the mediastinal contents, the animal being maintained with artificial respiration. The cervical sympathetic nerve was freed in the neck and sectioned peripherally.

Because it is difficult to determine variations in nervous activity in records of the summated action potentials from the many fibers of a nerve trunk, we have in general employed small twigs of the cardiac nerve split off from the main body. Single fibers of the cervical sympathetic nerve were obtained by careful dissection in a moist chamber until visual and aural examination of the amplified action potentials indicated that only a single functional unit remained active. In all experiments the animal was kept in a shielded room maintained near 37°C. and 100 per cent humidity.

Action potentials were suitably amplified with a capacity coupled amplifier and recorded with a General Electric oscillograph and a bromide paper camera. A few records were made with a cathode ray oscillograph. Simultaneous records of blood pressure were made in a number of experiments with an optical manometer connected with either the femoral or carotid arteries.

RESULTS. *Nature of activity in the cardiac nerve.* The spontaneous activity which may be recorded from the inferior cardiac nerve is typically grouped into smooth potential waves of relatively large amplitude (Bronk, Ferguson, Margaria and Solandt, 1936). These waves are frequently synchronous with the pulse and occur at a frequency of 2 or 3 a second. Such a pulse modulation of the discharge is dependent upon the integrity of the buffer nerves. Bronk (1934) has shown that it represents the tonic accelerator activity of the medullary cardiovascular centers, periodically inhibited by the discharge of pressure receptors located in the carotid sinus and aortic arch. The nature of this grouped activity in the inferior cardiac nerve is unaffected by removal of brainstem structures above the pons, and is in no wise dependent upon the hypothalamus (Bronk, Pitts and Larrabee, 1940).

Effect of hypothalamic stimulation on cardiac nerve discharge. Stimulation of the hypothalamus with low intensity, high frequency (150 per sec.)

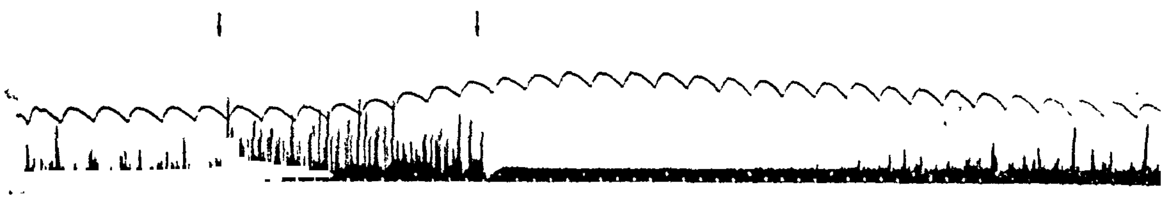

Fig. 1. Increased discharge of impulses in the cardiac sympathetic nerve during hypothalamic stimulation at a frequency of 150 per second, followed by inhibition of spontaneous discharge. Arrows mark beginning and end of the period of stimulation. Upper tracing, arterial blood pressure. Time, ⅕ second.

condenser discharges leads, after a brief latency of less than 0.1 second, to a marked increase in activity in the cardiac nerve. The abrupt onset of this activity as stimulation is begun (first arrow) and its equally abrupt cessation as stimulation is stopped (second arrow) is apparent from figure 1. In this experiment blood pressure began to rise after an interval of 1.5 seconds, continued to rise for 1 second after stimulation was stopped, and remained elevated for another 6 seconds or more. In contrast to the prolonged elevation of blood pressure, activity in the inferior cardiac nerve ceased abruptly as the stimulus was stopped, and for the succeeding 4 seconds all spontaneous discharge was abolished.

It is apparent that the postulated prolonged after-discharge from the hypothalamus following stimulation (Grinker and Serota, 1938) finds no support in our experiments on the anesthetized animal. Prolonged elevations of blood pressure which do occur in these experiments are obviously due to inertia of the effector system and not to sympathetic after-discharge.

Often, if the stimulus is prolonged or made more intense, a secondary

rise in blood pressure occurs some 10 or more seconds after the initial rise. This secondary rise, which may occur some seconds after the stimulus is stopped and at a time when all sympathetic activity is inhibited, is interpreted as being due to the release of adrenalin, described by Houssay and Molinelli (1925) and Magoun, Ranson and Hetherington (1937). In accord with the experience of Kabat, *et al.* (1935), the heart is but little

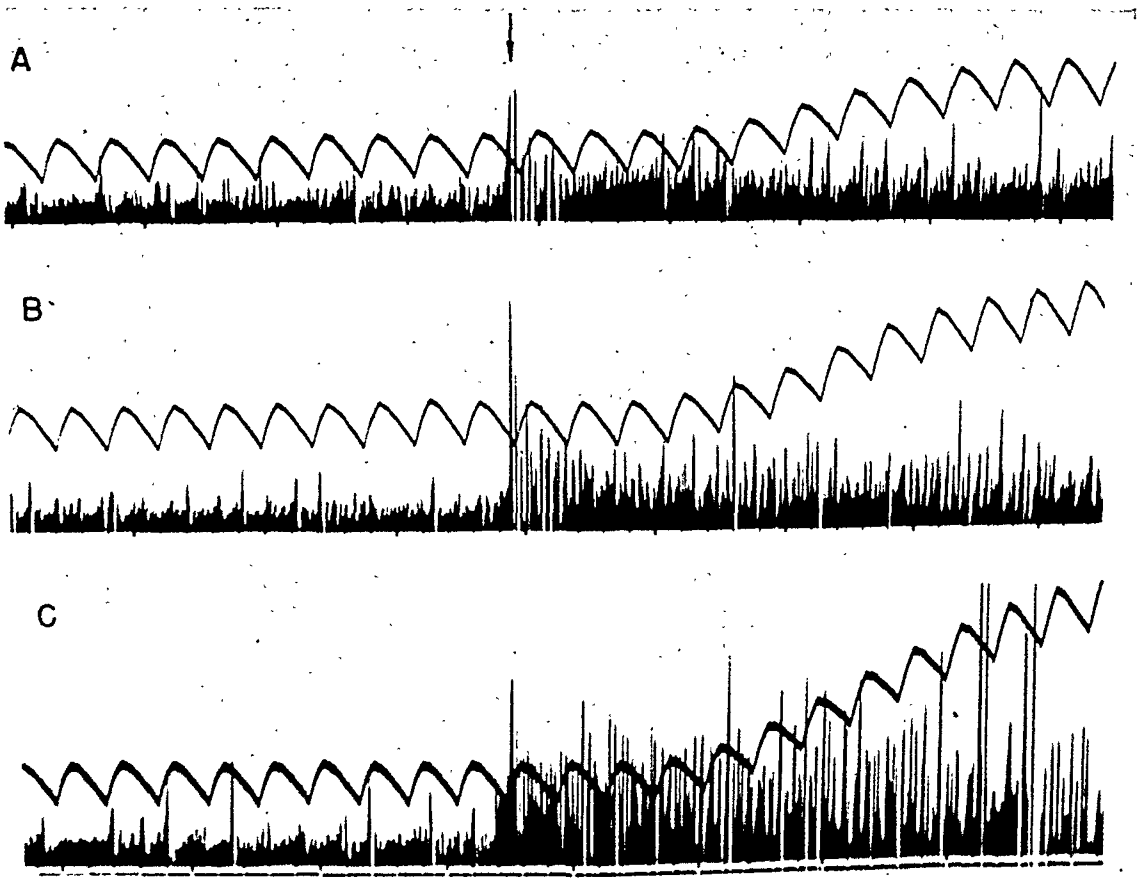

Fig. 2. Increased discharge of impulses in the cardiac sympathetic nerve and rise of blood pressure during hypothalamic stimulation at a frequency of 150 per second and relative intensities of 1 in A, 2 in B and 3 in C. Arrow marks the beginning of stimulation. Time, $\frac{1}{5}$ second.

accelerated, rarely more than 10 per cent, a fact no doubt correlated with the high heart rate and lack of vagal tone in the cat.

Factors controlling hypothalamic excitation and cardiovascular response. A. Stimulus intensity. An increase in intensity of hypothalamic stimulation accentuates those processes which have just been described. Figure 2 illustrates the results of stimulation of the hypothalamus at a constant frequency of 150 per second, but with increasing relative intensities of 1 in record A; 2 in record B; to 3 in record C. With increase in stimulus

strength, the discharge in the cardiac nerve progressively increased from A to C. The magnitude of the blood pressure rise and the steepness of its gradient increased in parallel manner with increasing stimulus strength.

Had the records of figure 2 been reproduced over a longer period, they would show from A to C a progressive increase in the time that spontaneous activity in the cardiac nerve was inhibited following cessation of stimulation. They would show an increased duration of the elevation of blood pressure, as well as an increased secondary rise in blood pressure, as a result of increased stimulus strength.

Records such as these illustrate well the effects of hypothalamic stimulation, but little concerning the mechanism by which they are achieved.

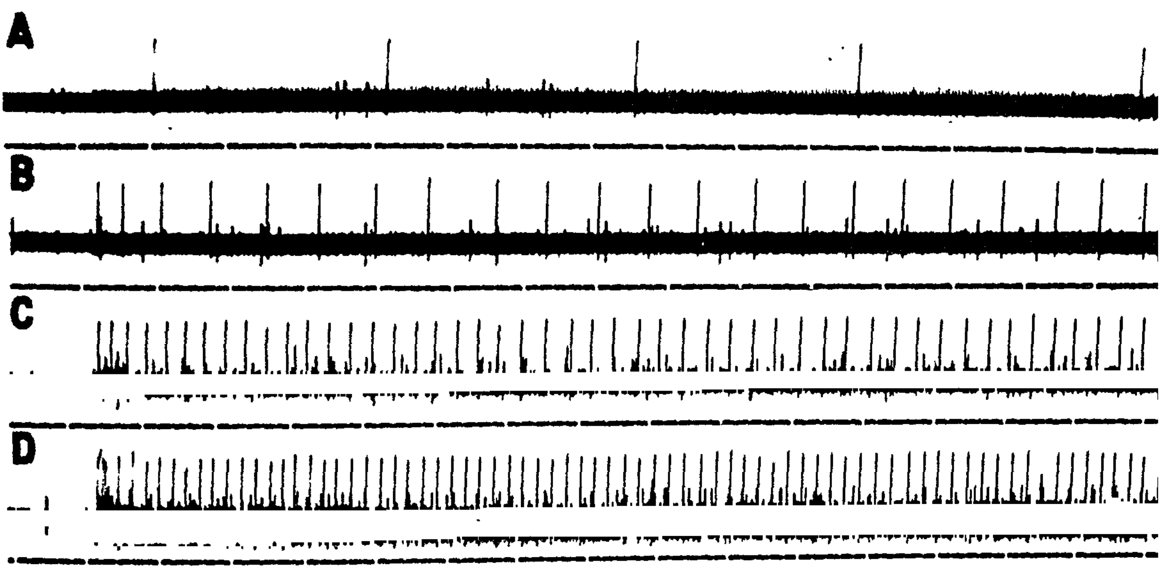

Fig. 3. Discharge of impulses in a single fiber of the cervical sympathetic nerve in response to hypothalamic stimulation at a frequency of 100 per second. Intensity progressively increased from A to D. Time, $\frac{1}{2}$ second.

It is readily apparent that the number of messages carried by a nerve to the heart or blood vessels is greatly increased by hypothalamic stimulation; but so many messages are being carried by so many different fibers, that the fundamental content of any one message is obscured by the activity of the mass.

Accordingly, we have studied the nerve impulses carried by single nerve fibers dissected from the cervical sympathetic trunk. Records from such a preparation are presented in figure 3. Instead of the random mass discharge observed in figures 1 and 2 from a many fiber preparation, the single unit here illustrated responded rhythmically and repetitively to hypothalamic stimulation. The story which such a unit tells is a simple one; its one variable is frequency of response. As a result of increasing

strength of hypothalamic stimulation, such a single unit responds with increasing frequency of discharge as illustrated in records A to D. In this experiment the frequency of hypothalamic stimulation was maintained constant at 100 per second and the intensity altered. With an increase in stimulus intensity of some 2.5 times, the response frequency of the neurone varied from about 1 in 2 seconds to 9 per second.

We have arbitrarily selected for all our studies, single fibers which were not spontaneously active. Such units, after a period of a second or so of adaptation, maintain a very constant frequency of discharge in response to maintained hypothalamic stimulation. If a period of a half minute or more intervenes between two periods of stimulation, the response frequencies

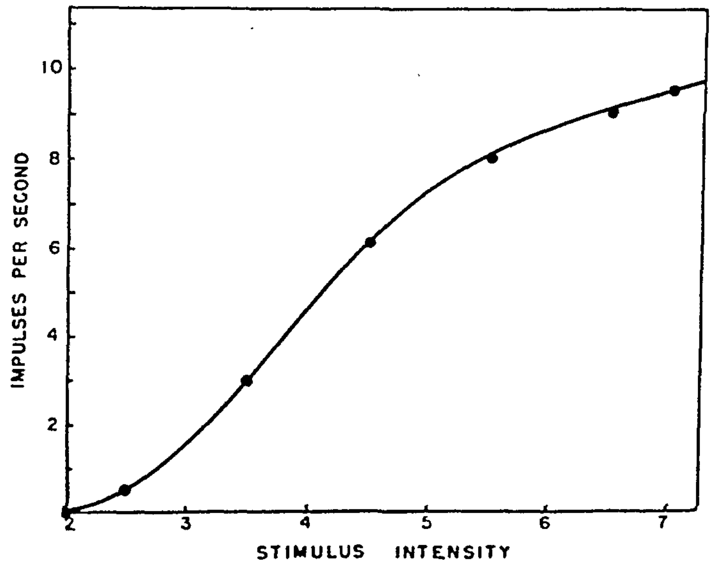

Fig. 4. The relation between frequency of discharge of impulses in a single fiber of the cervical sympathetic nerve and intensity of hypothalamic stimulation. Frequency of stimulation constant at 100 per second.

are reproducible. These facts make quantitation of our results in terms of impulse frequency an adequate method of presentation.

A plot of impulse frequency against stimulus intensity, taken from the entire series of records of which figure 3 is a part, is shown in figure 4. The relationship is smoothly curved and sigmoid in shape. The intensity units are arbitrary ones, but the highest intensity used is below that at which any significant motor response occurs, a limitation enforced by our methods of recording.

From these observations of the behavior of single fibers as the intensity of hypothalamic stimulation is increased, the records of figures 1 and 2 acquire added significance. The gross increase of activity observed in the cardiac nerve must, in part, be due to an increase in the number of impulses

carried by the constituent units making up that nerve. The temporal summation of those impulses at the effector organ produces a response which is graded according to impulse frequency, and hence according to intensity of hypothalamic stimulation. However, increase in intensity of hypothalamic stimulation not merely increases the frequency of discharge in a given unit, but also increases the number of active units as well, for their thresholds of excitation vary over a wide range. Examples of such increase in number of active units will be presented in a later section. Suffice it to say here, that magnitude of response in sympathetic activity is determined by variations in both the frequency of impulses in any one neural unit and in numbers of neural units active. Such a relation has been described by Adrian and Bronk (1928) for the gradation of somatic motor reflexes and by Bronk and Ferguson (1935) for the gradation of intercostal respiration.

B. *Placement of electrodes.* The intensity of hypothalamic excitation may be varied not only by alteration of stimulus strength, but also by changing the placement of the stimulating electrodes within the hypothalamus. The strengths used for hypothalamic stimulation have been low, as mentioned above, and spread of stimulus has been kept to a minimum by using bipolar electrodes, the tips of which were separated about 0.2 mm. The distance of current spread from the electrode tips is small under such conditions (Pitts, 1941).

In figure 5 is shown the response of two neurones, each identifiable by its characteristic spike potential. During each record, the hypothalamus was activated by the same low intensity stimulation at a frequency of 180 per second. From A to E, the electrode was progressively lowered through the hypothalamus in steps of 1 mm. Figure 6 A shows the position of the electrode at each level within the hypothalamus. To the right of the electrode track is given the frequency of discharge of the neurone whose spike potential is the larger; to the left is the letter identifying the level with a particular record of figure 5. In figure 6 B are presented the results of another experiment, in which a single fiber was activated from a number of levels on both the ipsilateral and contralateral sides of the hypothalamus. The relatively constant frequency of discharge to stimulation over the lower 3 mm. of the hypothalamus on both sides, coupled with the sudden decline in frequency as the electrode is raised above this level can only mean that the hypothalamic field, which is in functional relationship with a given preganglionic sympathetic neurone, is not only bilaterally distributed, but also has a considerable dorsoventral extent. This view is supported by a number of experiments in which the frequency of discharge of a given single preganglionic neurone has been determined for stimuli of various intensities applied to the two sides of the hypothalamus. A weak stimulus, which is just threshold for the side which is ipsilateral to the cervical

sympathetic neurone whose activity is being recorded, will usually excite that neurone if applied to the contralateral hypothalamus. If not, a slight increase in intensity will bring it to a threshold level.

The results presented so far may be most readily interpreted as indicating that a number of parallel pathways connect the hypothalamus with the

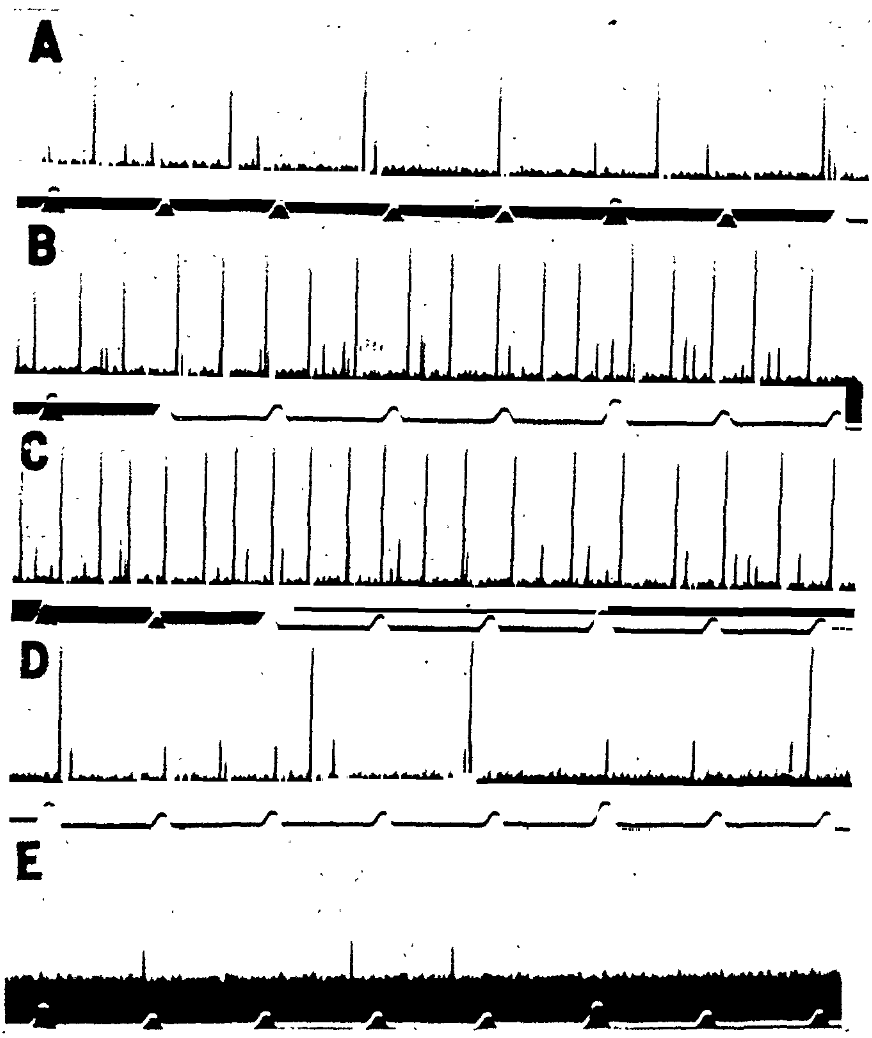

Fig. 5. The discharge of impulses in two fibers of the cervical sympathetic nerve in response to stimulation of the hypothalamus at succeeding dorso-ventral levels separated by 1 mm.

final preganglionic neurone located in the anterolateral column of the cord. An increase in intensity of hypothalamic stimulation increases the number of these pathways in action. A shift in the position of the stimulating electrode may bring a greater number of these pathways within the zone of influence of the stimulus, and thus increase the number in action. The discharge frequency of the final common neurone is, among other things,

some function of the number of parallel pathways in action. These experiments shed no light on the anatomical makeup of these pathways, i.e., upon the number of synapses between hypothalamus and final common path; but that there is extensive decussation within the system is evident.

C. *Stimulus frequency.* The effect of increasing the frequency of hypothalamic stimulation upon the discharge in the inferior cardiac nerve and upon the associated rise in blood pressure is similar to that of increasing the intensity of stimulation. In fact, records identical in all respects to

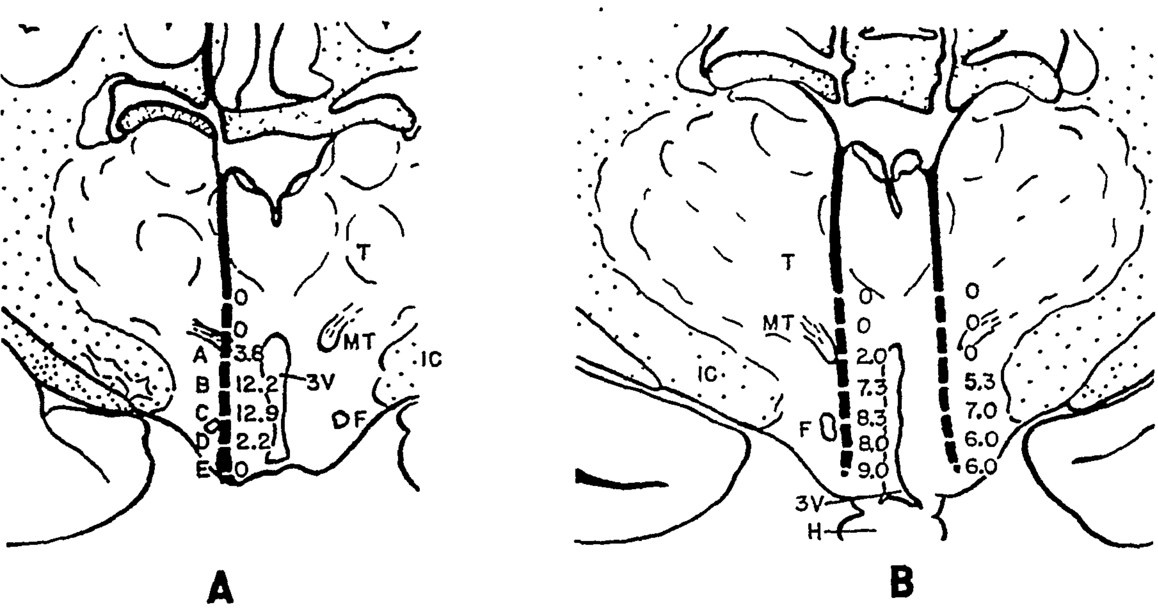

Fig. 6A. Tracing of a stained section through the diencephalon of the cat. The heavy vertical line indicates the track of the stimulating electrodes, the interruptions of the line the position of the bare electrode tips. Letters A to E on the left correspond to the points which were stimulated during preparation of the similarly lettered records of figure 5. The numbers to the right of the electrode track give the frequency of discharge in impulses per second of the cervical sympathetic neurone of large spike potential

B. Location of stimulating electrodes in a similar experiment in which a single cervical sympathetic neurone was caused to discharge at the indicated frequencies by stimulation at a number of levels on both sides of the hypothalamus.

those shown in A, B and C of figure 2 have been obtained by stimulation of the hypothalamus with frequencies of 50, 100 and 150 per second, maintaining the intensity constant. The degree of activity in the cardiac nerve during hypothalamic stimulation and the duration of the period of inhibition of spontaneous activity after stimulation increase in proportion to the frequency of stimulation. Likewise, the magnitude of the rise in blood pressure and the length of time that it remains elevated show a similar proportionality to frequency of stimulation.

The response of single fibers of the cervical sympathetic to an increase

in frequency of hypothalamic stimulation is qualitatively similar to that shown in figure 3 for an increase in stimulus intensity. Quantitatively, however, the results differ. In the same experiment from which figures 3 and 4 were taken, the stimulus intensity was maintained constant and the frequency varied. A plot of the response frequency of the single neurone against the stimulus frequency applied to the hypothalamus yields a relationship which is remarkably linear, figure 7. The frequency of firing of the neurone in this experiment maintained an essentially constant ratio to the frequency of hypothalamic stimulation, the neurone firing once to approximately every twenty-fifth stimulus throughout the range investigated.

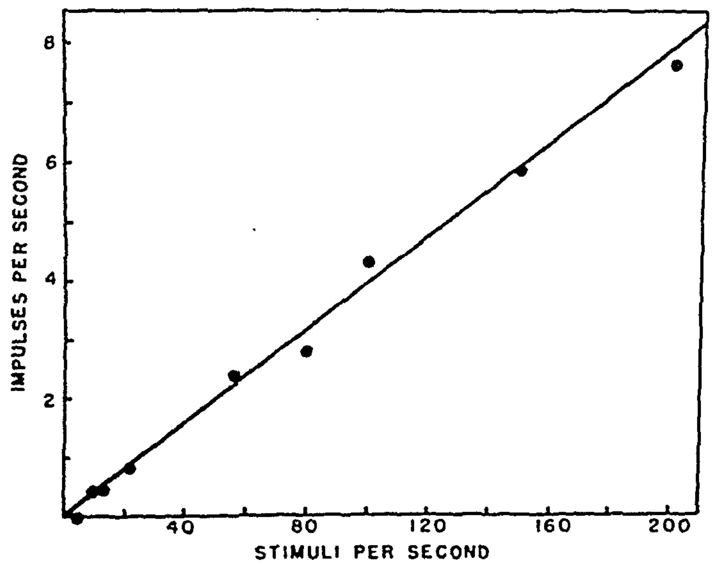

Fig. 7. The relation between frequency of discharge of impulses in a single fiber of the cervical sympathetic nerve and frequency of hypothalamic stimulation. Intensity of stimulation constant.

Not only does an increase in stimulus frequency increase the activity of any single unit, but it also brings into activity new units, not active at the lower stimulus frequency. Figure 8 illustrates the effect of increasing the frequency of hypothalamic stimulation on the number of neurones responding in a multifiber preparation. In records A, B and C the stimulus frequencies were 8.5, 18 and 43, respectively, the intensity being maintained constant. In records A and B only the neurone characterized by the high, thin spike potential responded. In record C, at the higher frequency of stimulation, the response of this same neurone may again be identified, but a number of other neurones whose spike potentials are different in form also responded. Facilitation at some point in the descending pathways from the hypothalamus probably accounts for this radiation of excitation over channels not responsive to volleys of lower frequency.

The results presented above indicate not only that multiple pathways descending from the hypothalamus converge on single effector neurones but also that each descending pathway establishes connections which diverge and impinge on many effector neurones. Experiments presented in succeeding pages indicate that important loci of convergence and divergence lie in the medullary sympathetic centers.

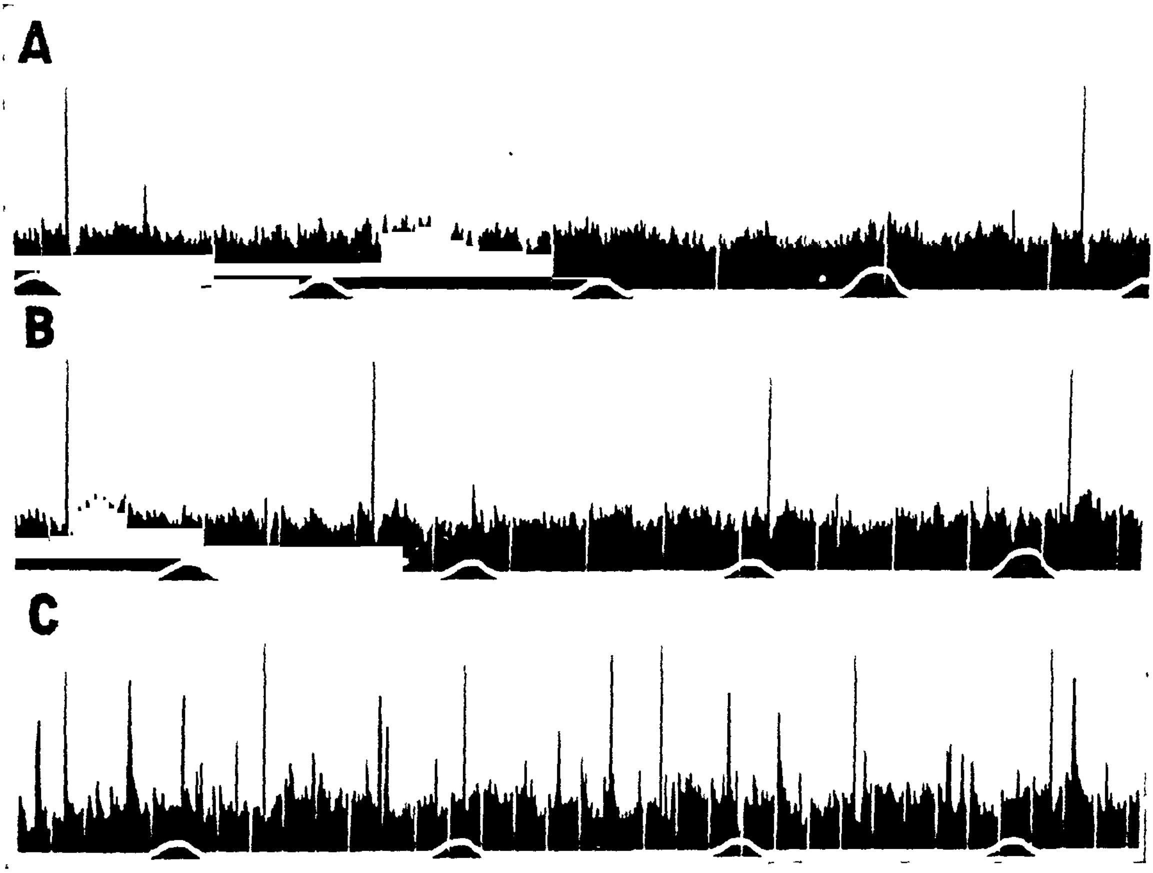

Fig. 8. Discharge of impulses in a multifiber preparation of the cervical sympathetic nerve during stimulation of the hypothalamus at frequencies of 8.5, 18 and 43 shocks per second. In record C at the higher rate of stimulation several fibers respond in addition to the one active in A and B.

D. *Reversal of response from change in frequency of stimulation.* The responses to hypothalamic stimulation which have been described in the preceding paragraphs, all support the generally accepted view that the hypothalamus serves as a center for the regulation of sympathetic function. Cushing (1931) first ascribed importance to the hypothalamus as a regulating center for parasympathetic functions as well. Beattie (1932) and Fulton (1932) maintained that the hypothalamus could be divided functionally into an anterior parasympathetic portion and a posterior sympathetic portion. Subsequent work reviewed by Ranson and Magoun

(1939) and Ingram (1939) as well as our own has given little support to such a view. We have found no difference in the type of sympathetic response on stimulation of the supraoptic, tuberal or mammillary portions of the hypothalamus although the lateral and more caudal parts seem to be somewhat more reactive. We have not, however, studied parasympathetic activity.

An interesting reversal from a pressor to a depressor type of response has been observed occasionally on stimulation of the hypothalamus at low frequencies. The records presented in figure 9 A and B are typical of such an experiment. Record A shows the usual pressor response and the increased activity in the inferior cardiac nerve from low intensity

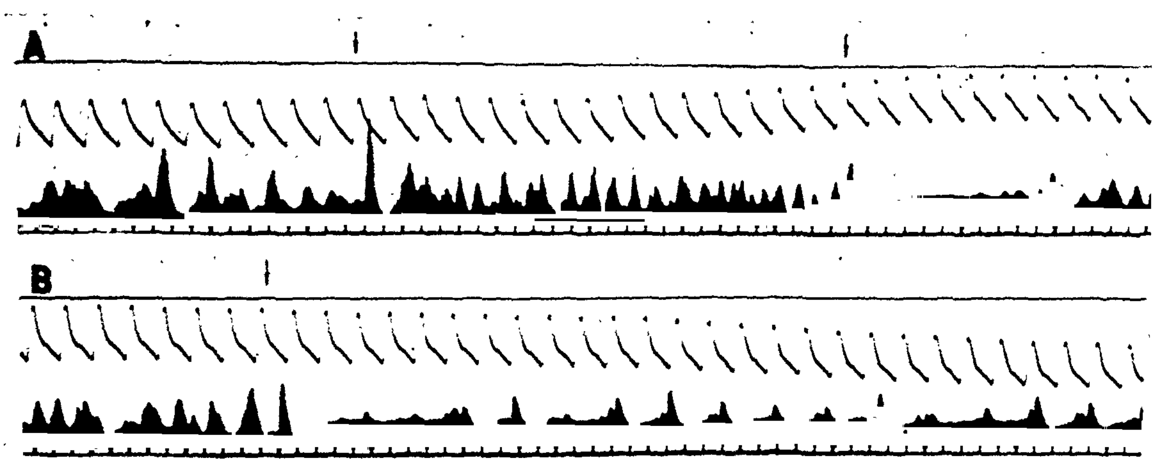

Fig. 9. Reversal from a pressor to a depressor response on lowering the frequency of hypothalamic stimulation. A. Stimulation of the hypothalamus at 20 per second; sympathetic excitation and rise of blood pressure. B. Stimulation at 2 per second; sympathetic inhibition and fall of blood pressure. Arrows indicate beginning of stimulation and also the end in A. Time, ½ second.

hypothalamic stimulation at a frequency of 20 per second. In record B the intensity of stimulation and the placement of the electrodes were unchanged but the frequency of stimulation was reduced to 2 per second. Stimulation at this lower frequency led to a partial inhibition of spontaneous activity in the cardiac nerve and a gradual fall in blood pressure. Both the slight slowing of the heart (5 per cent) and the fall in blood pressure were unchanged by section of the vagi.

Although this reversal from a pressor to a depressor response has been noted in several experiments, we have not been able to repeat it at will. There seems to be no characteristic part of the hypothalamus from which this reaction may be elicited. Hare and Geohegan (1939) and Berry and Hodes (1941) have noted this same reversal on lowering the frequency of hypothalamic stimulation.

There is a noteworthy similarity between the response reversal from hypothalamic and from peripheral nerve stimulation. In both, the lower frequencies favor the depressor type of response, the higher frequencies the pressor type. Whether this response reversal results from activation of two fiber systems in the hypothalamus as has been postulated for peripheral nerve (Ranson, 1916), or is an expression of the effects of volleys of impulses of different frequencies over the same pathways is unknown. Kabat *et al.* (1935) maintain that depressor pathways originating in the preoptic and more cephalic regions of the forebrain descend through the hypothalamus. It is possible that such pathways are more easily excited by low frequency stimulation than are the pressor ones, though we have no evidence on this matter. These experiments suggest, however, that some of the so-called parasympathetic responses which have been described as resulting from hypothalamic stimulation actually result from inhibition of tonic sympathetic outflow.

Relation between frequency and intensity of hypothalamic stimulation. Since a change of position of the stimulating electrodes within the hypothalamus may alter the number of active projections which impinge upon any given sympathetic neurone, it is unlikely that any two neurones in the same or different preparations will ever behave identically to a given stimulus intensity or frequency. The maximum frequency of discharge of sympathetic neurones, at the highest intensity and frequency of hypothalamic stimulation which can be applied without causing any considerable degree of somatic motor response, varies between 10 and 50 impulses per second. A part of this variation is undoubtedly due to differing numbers of pathways to that neurone which lie within the zone of influence of the stimulus. Another factor in this variation may be differences in excitability of the several neurones. Although there are these marked differences in the quantitative behavior of sympathetic neurones, the qualitative aspects of their behavior in relation to changes of frequency and intensity of hypothalamic stimulation are remarkably reproducible.

In 7 experiments in which the relationship between impulse frequency and stimulus intensity was examined, it was found to have the characteristic sigmoid form of figure 4, although the maximum impulse frequency varied between 10 and 50. Similarly in 8 experiments a relationship was noted between impulse frequency and stimulus frequency which was linear over a considerable part of the range, though here again the peak frequencies varied over wide limits.

If it is true that the peak frequencies of impulse initiation in these experiments are related to the number of pathways excited as well as the frequency of their excitation, then an increase in intensity of stimulation should not alter the general character of the relationship between impulse frequency and stimulus frequency, but merely change magnitudes. In figure 10,

records A, B, C and D, E, F, the same three frequencies of stimulation were applied to the hypothalamus. In the A to C group, the relative intensity was 1; in the D to F group, it was 3. That an increase in intensity at each

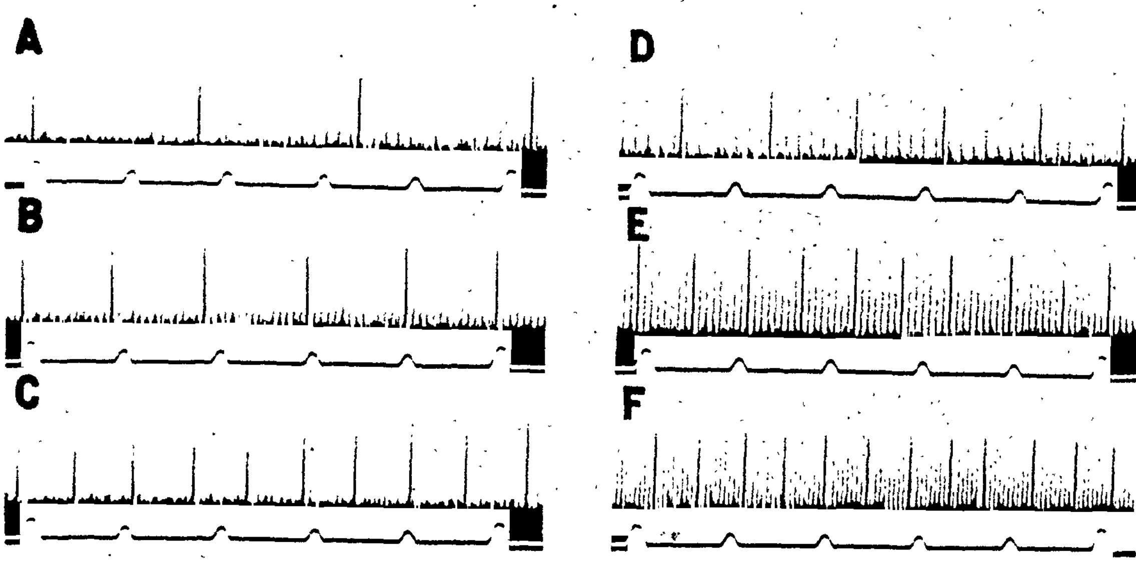

Fig. 10. The discharge of impulses in a single fiber of the cervical sympathetic nerve during hypothalamic stimulation at 3 frequencies and 2 intensities. Left column, A to C, intensity of 1; right column, D to F, intensity of 3. Frequency of upper row, 38; middle row, 68; lower row, 100 per second. Time, $\frac{1}{5}$ second.

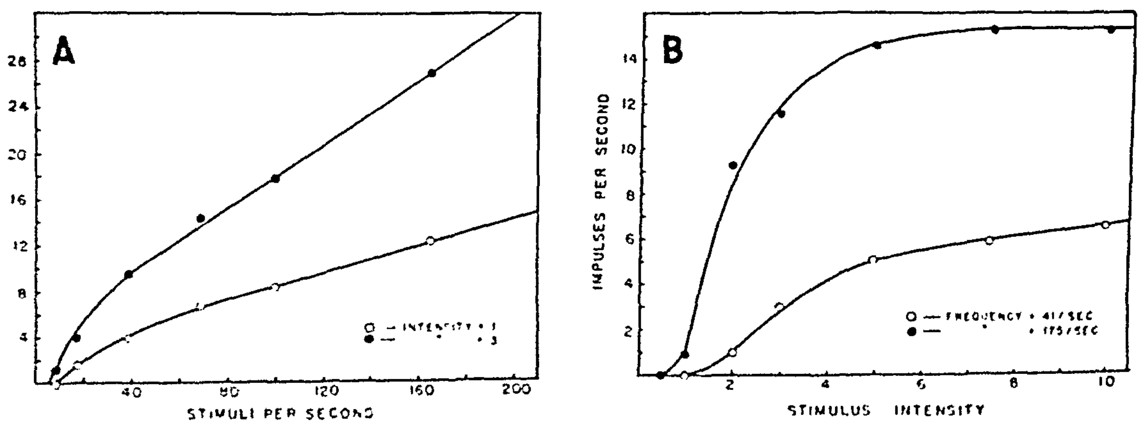

Fig. 11A. The relation between frequency of discharge of impulses in a single fiber of the cervical sympathetic nerve and frequency of hypothalamic stimulation at relative intensities of 1 and 3.

B. Similar relationship between frequency of discharge and intensity of stimulation at stimulus frequencies of 41 and 175 per second.

frequency of stimulation produces an increase in impulse frequency is apparent by comparing A, B, C (low intensity), with D, E, F (higher intensity). In figure 11 A the complete data of such an experiment are

presented graphically. The relation between impulse frequency and stimulus frequency at each intensity is linear throughout the greater part of the range, though the slopes differ. The peak frequency of this neurone varies between 12 and 27, depending on the numbers of pathways excited, yet the general relationship is unchanged.

One difference in this relationship which we have noted between different neurones is illustrated by comparing figures 7 and 11 A. An extrapolation of the linear part of the relation in figure 7 passes through the origin; in figure 11 A it strikes above the origin. It seems plausible to assume that while the two neurones do not fire spontaneously, the one whose behavior is illustrated by figure 10 A may be receiving subliminal excitation from another source than the hypothalamus, while the neurone of figure 7 does not. Such subliminal excitation summing with hypothalamic excitation might produce a frequency of firing at each stimulus frequency higher by a constant amount than would be predicted.

As illustrated by figure 11 B, a change of stimulus frequency from 41 per second to 175 per second does not seriously alter the form of the relationship between impulse frequency and stimulus intensity, although the peak values vary between 7 and 15 impulses per second as a result of increasing the rate of stimulation of a constant number of pathways.

Integration of hypothalamic cardiovascular responses. A. *Antagonism between buffer reflexes and hypothalamic cardiovascular responses.* Stimulation of the hypothalamus with even low intensity stimuli disturbs to a considerable degree the delicate regulation which maintains blood pressure and heart rate within normal limits. Some aberration of the normal is prerequisite for the hypothalamus to exercise its function of adjustment to emotional and thermal factors in the environment, yet the basic need for regulation remains unchanged. Since hypothalamic activity and activity of the buffer reflexes are in direct opposition to one another, the degree of their interplay is an important factor in analyzing cardiovascular regulation.

Bronk and Stella (1932) have shown that pressure sensitive endings within the walls of the carotid sinus and aorta discharge impulses whose frequency is proportional to the pressure. A rise in pressure increases the frequency of discharge and brings into activity new endings which did not respond at the lower pressures. These impulses carried to the medullary cardiovascular centers over the nerves of Hering and the depressor nerves produce an inhibition of tonic sympathetic outflow to the heart and blood vessels which results in a lowering of pressure toward normal (Bronk, Ferguson and Solandt, 1934).

Evidence of the activity of the buffer mechanism is seen in figure 1. The tonic activity in the inferior cardiac nerve, which is evident at normal pressure levels prior to hypothalamic stimulation, is completely abolished

during the period of elevated pressure following the cessation of stimulation. Records from single fibers of the depressor nerve clearly indicate that the pressure sensitive endings within the aortic arch respond to the rise in blood pressure produced by hypothalamic stimulation with an increasing frequency of discharge. Central stimulation of the depressor nerve at a comparable frequency causes inhibition of the tonic sympathetic outflow over the cardiac nerve.

Such experiments indicate the extent to which the buffer reflexes operate in returning conditions to normal following hypothalamic stimulation. Experiments of another type, however, must be performed to show whether the buffer reflexes act to oppose the changes induced during the period of hypothalamic stimulation. If brief bursts of high frequency stimuli are applied to the hypothalamus at intervals of 1 second, each burst of stimuli is

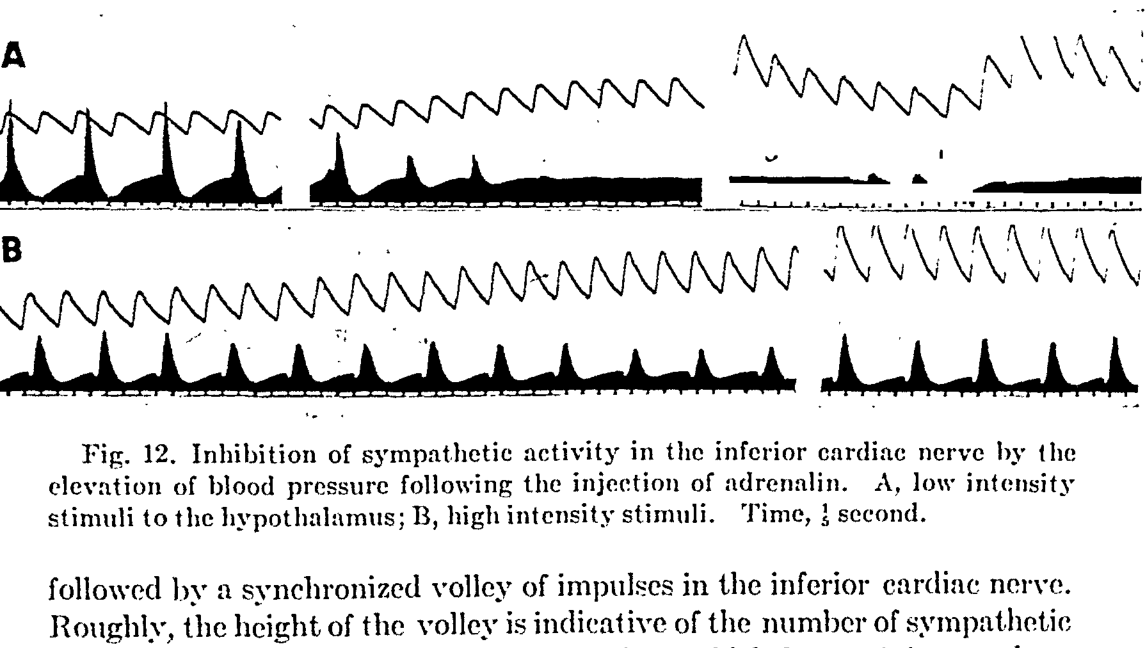

Fig. 12. Inhibition of sympathetic activity in the inferior cardiac nerve by the elevation of blood pressure following the injection of adrenalin. A, low intensity stimuli to the hypothalamus; B, high intensity stimuli. Time, ⅕ second.

followed by a synchronized volley of impulses in the inferior cardiac nerve. Roughly, the height of the volley is indicative of the number of sympathetic neurones responding. In the experiment from which figure 12 A was taken, bursts of stimuli of low intensity were applied to the hypothalamus once a second throughout the entire record. Adrenalin was injected during the interval indicated by the first break in the record. As the blood pressure rose, the volleys decreased progressively in height, until inhibition became complete, although stimulation continued unchanged. In the last part of the record, a fortuitous fall in blood pressure permitted a volley to escape, indicating most clearly that inhibition of the periodic hypothalamic drive resulted from the rise in pressure. The experiment was then repeated in an identical manner except that the intensity of the bursts of hypothalamic stimulation was increased. Record B illustrates that the buffer reflexes, brought into play by the rise in blood pressure could not completely inhibit

the volleys initiated by the more intense hypothalamic stimuli, though they could reduce for a time the number of neurones responding.

An extension of this principle is illustrated by the records of figure 13. A single neurone of the cervical sympathetic was caused to fire repetitively by high frequency, very low intensity stimulation of the hypothalamus,

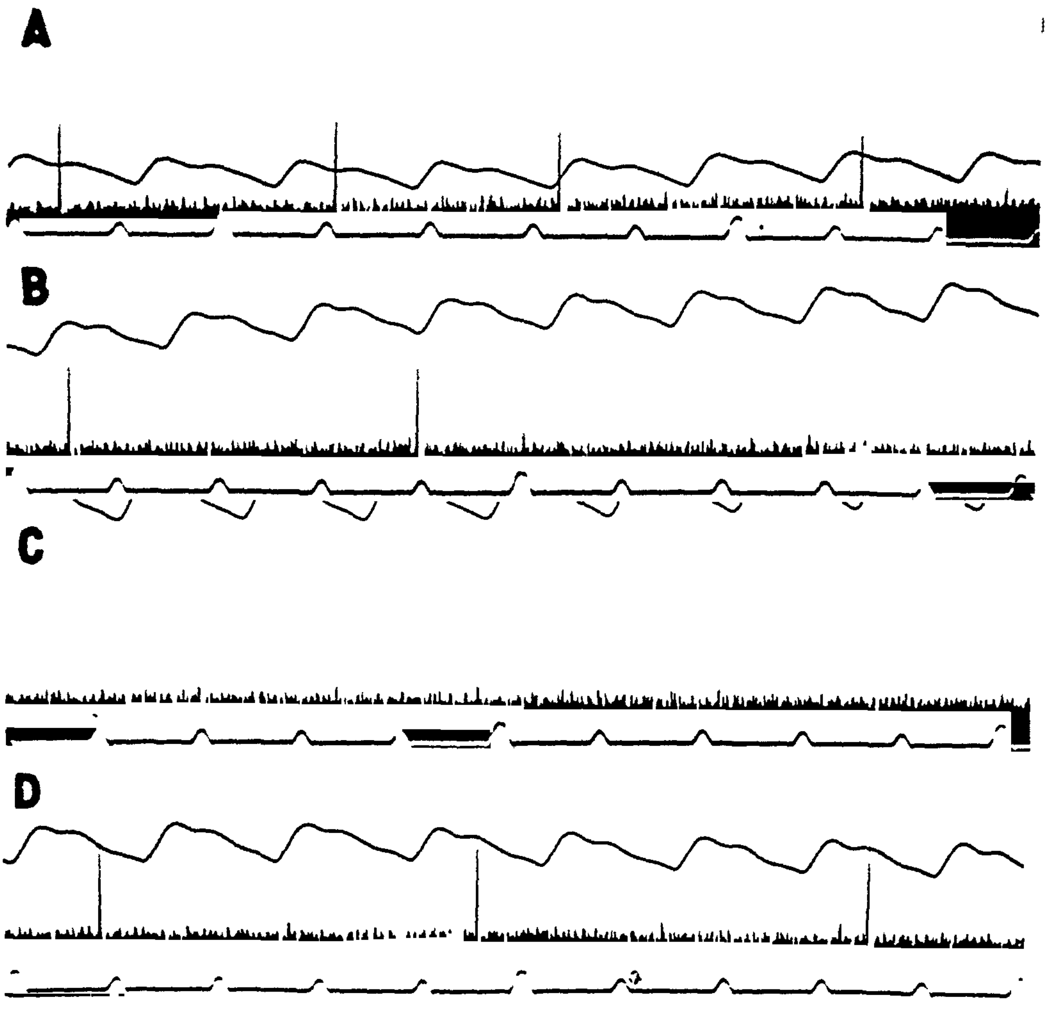

Fig. 13. Slowing and inhibition of discharge in a single fiber of the cervical sympathetic nerve by the elevation of blood pressure following the injection of adrenalin. Between records A and B, adrenalin was administered intravenously. Time, $\frac{1}{5}$ second.

maintained constant throughout the entire series of records. In the interval between records A and B, adrenalin was given intravenously, and with the rise in pressure, the response frequency diminished until the neurone ceased to fire, record C. With return of pressure toward normal, the neurone again began to fire repetitively, record D. Stimulation of the hypothalamus with more intense stimuli caused this neurone to fire

more rapidly, and under these circumstances, a rise of blood pressure produced a negligible slowing of the response.

These experiments indicate conclusively that the buffer reflexes do act to oppose a rise of pressure during the period of hypothalamic stimulation. Furthermore, it is clear that activation of the buffer reflexes interposes a relative block between hypothalamus and efferent sympathetic outflow. If the buffer reflexes are activated maximally, and if the intensity of hypothalamic stimulation is low, the block can be an absolute one. Or it may be only a relative block, diminishing the number of responding efferent motor neurones and the frequency at which they respond if the intensity of hypothalamic stimulation is high. Such results can best be interpreted on the basis of a synaptic break in the descending hypothalamic pathways, at which the afferent buffer nerves may exert an inhibitory action.

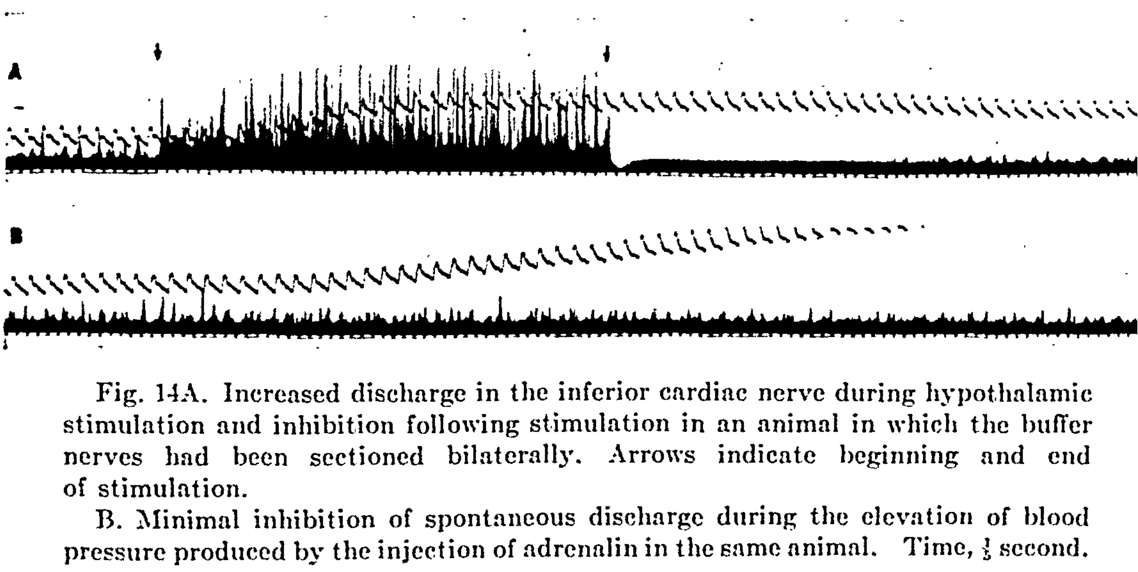

Fig. 14A. Increased discharge in the inferior cardiac nerve during hypothalamic stimulation and inhibition following stimulation in an animal in which the buffer nerves had been sectioned bilaterally. Arrows indicate beginning and end of stimulation.

B. Minimal inhibition of spontaneous discharge during the elevation of blood pressure produced by the injection of adrenalin in the same animal. Time, ⅕ second.

A factor, other than buffer reflex activation, also operates to produce the inhibition of spontaneous sympathetic activity which follows hypothalamic stimulation. The operation of this factor is clearly illustrated by hypothalamic stimulation in an animal in which the buffer nerves have been sectioned. Figure 14 shows that the inhibition of spontaneous activity following hypothalamic stimulation is marked (record A) even though the carotid sinus and depressor nerves had been sectioned bilaterally. The completeness of removal of the pressure sensitive afferents is illustrated by record B. The administration of intravenous adrenalin produced a considerable rise in blood pressure, yet there was only a minimal degree of inhibition of spontaneous sympathetic activity. The inhibition following hypothalamic stimulation in this experiment cannot be due to the rise in blood pressure. It appears to result from a diminished excitability of

the sympathetic centers following intense activity. An analysis of the time course of these excitability changes is the subject of another communication.

B. *Synergism between buffer reflexes and hypothalamic cardiovascular responses.* The tonic discharge of impulses in the inferior cardiac nerve commonly shows a marked pulse modulation. This synchronization of sympathetic volleys with the pulse depends upon the integrity of the buffer nerves, for when these nerves are sectioned, the volleys occur at random and more rapid frequencies. Figures 1 and 2 show that stimulation of the hypothalamus with high frequency stimuli of moderate intensity causes the discharge of impulses in volleys of random and relatively high frequency. The buffer nerves obviously are exerting no very marked control over the increased sympathetic activity induced by such intensities of stimulation. If, however, the hypothalamus is stimulated at low intensity with high frequency shocks, the moderately increased sympathetic activity

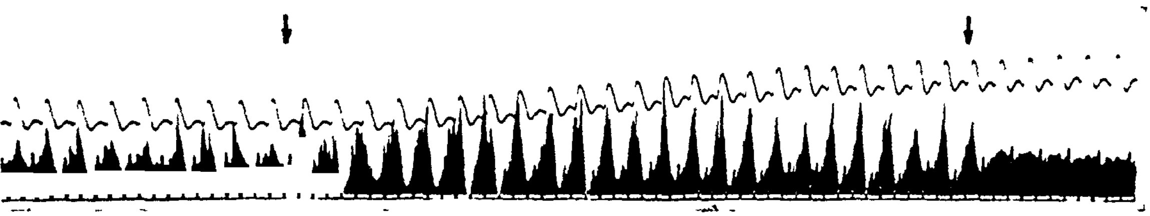

Fig. 15. Groups of sympathetic impulses in the cardiac nerve synchronous with the pulse. Increase of this grouped discharge during stimulation of the hypothalamus at 100 per second. Arrows indicate beginning and end of stimulation. Time, $\frac{1}{5}$ second.

may be shown to be checked and controlled by the buffer reflexes much as is the normal spontaneous activity. Figure 15 illustrates such synergistic action between the buffer reflexes and activity induced by low intensity hypothalamic stimulation. The first part of the record illustrates the spontaneous tonic sympathetic discharge synchronous with the pulse. During the interval marked off by the arrows, low intensity high frequency stimuli were applied to the hypothalamus. Stimulation results in both a rise in blood pressure and an increase in sympathetic activity in the cardiac nerve. But most striking is the fact that this activity is an accentuation of the normal, showing an even more definite pulse modulation. The conclusion is inescapable that such moderate increases in sympathetic activity induced by hypothalamic stimulation are woven into the normal pattern of sympathetic cardiovascular response without serious disarrangement of regulation.

C. *Summation of hypothalamic and peripheral nerve pressor responses.* The pressor response obtained on central stimulation of a peripheral sensory nerve is familiar to every student of physiology. That it may be

obtained in the decerebrate animal and is independent of structures above the pons for its central integration is similarly common knowledge. The central course of the pressor reflex pathway in the tract of Lissauer, integration at a medullary level, and efferent outflow through the ventrolateral columns of the cord were demonstrated by Ranson (1916).

We have observed on stimulation of a sensory nerve an increase in activity in the cardiac nerve and a rise in blood pressure which is qualitatively similar to that observed on hypothalamic stimulation. Thus central stimulation of the sciatic, femoral or peroneal nerves causes an abrupt increase in activity in the cardiac nerve, a rise in blood pressure after a latency of more than second, and following stimulation, an inhibition of spontaneous discharge in the nerve which lasts for the duration of the elevated blood pressure. The differences between the peripheral nerve pressor response and the response to hypothalamic stimulation are chiefly quantitative. In our experience the pressor response from nerve stimulation is variable in the anesthetized animal, unobtainable in some, and always less in magnitude than that obtained on hypothalamic stimulation. The results obtained, however, by simultaneous stimulation of the hypothalamus and a peripheral sensory nerve emphasize the essential similarity between the pressor responses elicited from the two sources. If strengths of stimuli are so adjusted as to produce comparable responses from hypothalamic and nerve stimulation, simultaneous stimulation leads to a response qualitatively identical to that from the two sources singly, but of a magnitude equivalent to their sum.

The foregoing observations indicate that hypothalamic activity is not permitted, unchecked, to disturb the balance of cardiovascular regulation. Nor, on the other hand, is buffer reflex adjustment so inflexible, that deviations of large magnitude are impossible. Rather, these experiments suggest that there is operative a delicate system of checks and balances which permits a deviation from normal directly related to the intensity of hypothalamic activity and inversely related to the degree of disturbance caused thereby. The buffer reflexes exert a control over sympathetic outflow induced by hypothalamic activity much as they do over spontaneous activity originating within the medullary centers. If the increase in sympathetic activity is slight, it is modulated and fitted into the same pattern as is the normal. If the increase is great, the buffer reflexes, no longer able to modulate it, at least hold it in check by a relative block at the medullary level. This block may operate to diminish both the number of sympathetic neurones responding and the frequency of response of any given neurone. The essential similarity between the pressor response from hypothalamic and sensory nerve stimulation, and the demonstration that they are capable of summation, suggests that under certain circumstances the two may show mutual re-enforcement. Such re-enforcement might

prove of value in activities where there is association of painful stimuli with the affective state.

Characteristics of the excitatory process. The factors so far considered as determining the response of sympathetic neurones to hypothalamic stimulation have been concerned with the more gross overall excitability relationships. However, if one examines certain experiments more closely, details of relationships appear. A single stimulus applied to the hypo-

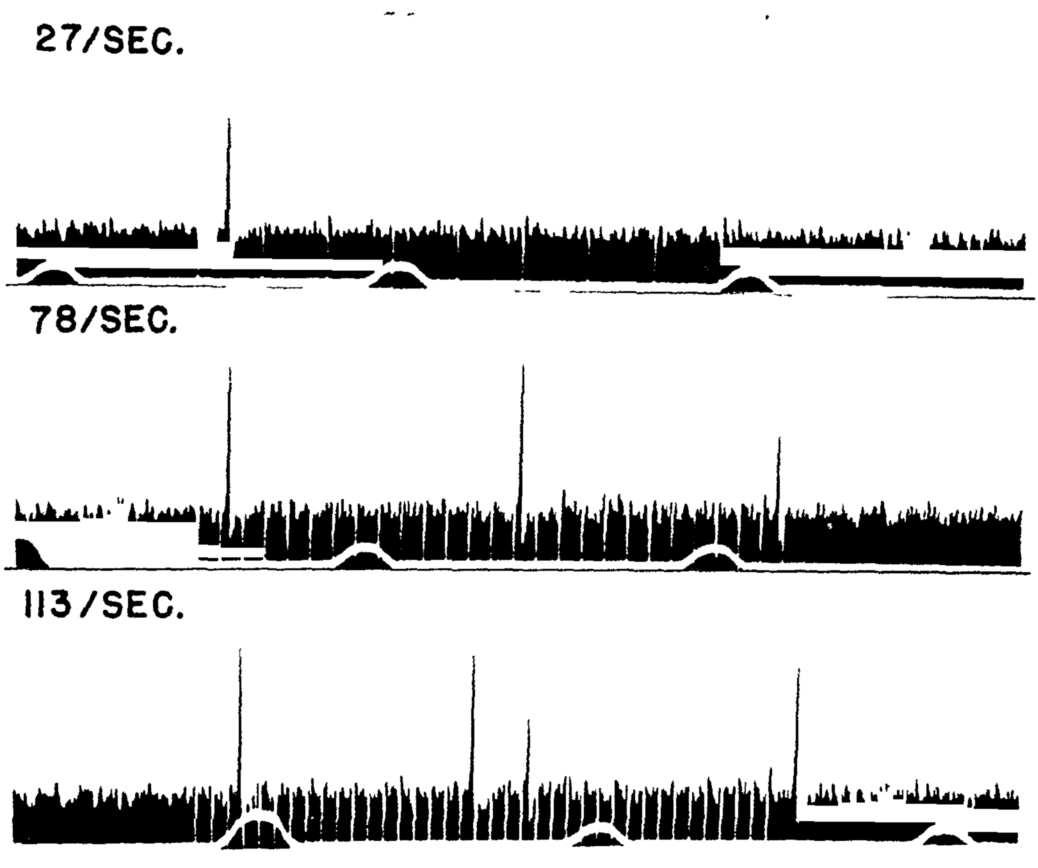

Fig. 16. Discharge of impulses in two fibers of the cervical sympathetic nerve in response to stimulation of the hypothalamus at the indicated frequencies. Time, $\frac{1}{5}$ second.

thalamus sets up a single volley of descending impulses whose magnitude, i.e., the *number* of participating pathways, depends at any one placement of the electrodes upon the stimulus strength. Such a volley of impulses may or may not cause a particular neurone of the cervical sympathetic to discharge an impulse. Most neurones which we have studied do not respond to single volleys of ordinary intensity, though some do. The response of both types of neurones to repetitive volleys is illustrated by figure 16. The neurone characterized by the larger spike potential fires in response to a single volley. The neurone characterized by the smaller

spike potential fires only after the summated effects of some 20 or more volleys. That there is no fundamental difference between these two neurone types is indicated by the fact that a neurone not responding to a single volley may be caused to do so by introducing into the hypothalamus high frequency subliminal stimuli through a second pair of electrodes.

Figure 3 illustrates a second principle, namely, the number of volleys which must be summated to fire a particular neurone depends upon the stimulus strength. In record A of figure 3, 0.43 second of hypothalamic stimulation at a frequency of 100 volleys per second was required before

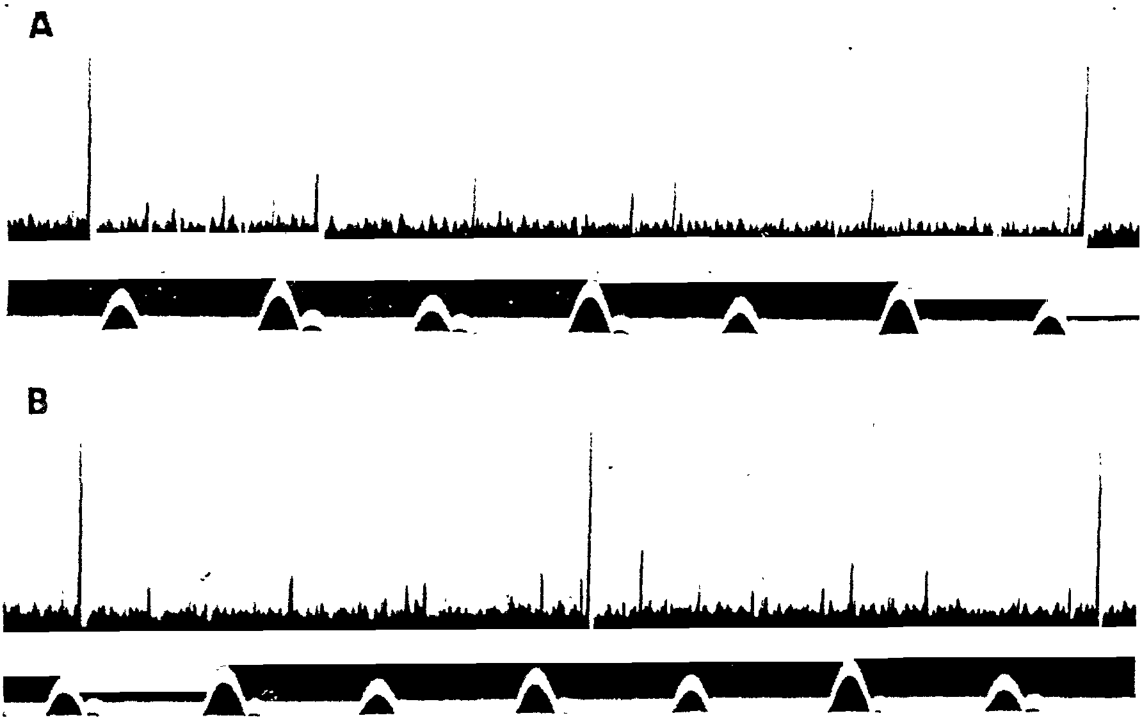

Fig. 17. Discharge of impulses in a single fiber of the cervical sympathetic nerve in response to stimulation of the hypothalamus at 5 and 13 per second. The stimuli are indicated by the faint upward deflections at regular intervals throughout each record. An impulse appears after the first and last stimuli in A. Three impulses are recorded in B. Time, 1/5 second.

the neurone gave its initial response. In record B an increase of 35 per cent in the intensity of the stimulus reduced the time to the first response to 0.03 second.

Figure 17 illustrates a third principle, namely, that independent of the number of volleys which must summate before a neurone fires, the neurone fires in response to or is triggered by a particular volley. There is a relatively constant latency in this experiment of 18 to 22 msec. from the start of a particular volley of impulses in the hypothalamus, to the arrival of a particular impulse in the cervical sympathetic at the point of recording in the neck.

Figures 3 and 16 also illustrate a further point of interest. The time from the beginning of stimulation to the first impulse is always less than the time from the first to the second impulse, often considerably less. Furthermore, the intervals between succeeding impulses tend to lengthen. In consequence of these changes, the neurone fires most rapidly at the beginning of stimulation, adapts to a lower frequency over the first second, and then maintains a frequency which only slowly declines over the remaining period of stimulation.

The observations just cited may be qualitatively described in terms used in studies on spinal reflexes (Creed, Denny-Brown, Eccles, Liddell, Sherrington, 1934) and on ganglionic transmission (Eccles, 1937), though it must be emphasized that our use of the terminology implies nothing as to the fundamental mechanisms involved. Thus the initial firing of the sympathetic neurone in response to hypothalamic stimulation depends on the building up of a central excitatory state by repetitive volleys from the hypothalamus, to a critical level, at which a single volley (detonator) triggers the mechanism and fires the neurone. Once the neurone has fired, the subsequent behavior of the system depends in part upon the average level of excitatory state maintained by the hypothalamic volleys, and in part upon the time course of recovery of excitability, a subject to be treated in another communication. In fact the steady state, which is characterized by repetitive firing of the cervical sympathetic neurone at a constant frequency to maintained hypothalamic stimulation, may be most readily visualized as a balance between these two factors.

The rate at which the excitatory state is initially built up and the final average level it maintains are directly related to the frequency of the hypothalamic volleys, and the number of pathways carrying volleys. The time for recovery of excitability, however, is directly related to the frequency at which the system fires. It follows that the increased frequency of firing resulting from increase in frequency or intensity of hypothalamic stimulation is attained by maintaining a higher average level of central excitation capable of exciting earlier in the recovery cycle. The period of rapid decline in discharge frequency at the start of stimulation finds explanation in summation of subnormality requiring a progressively increasing recovery time, which after a second or less becomes stabilized.

If we attempt to pass from the general to the specific in explanation of the above phenomena, no one incontrovertible fact enables us to choose between several current theories. The excitatory state which in our experiments shows such definite temporal summation can logically be explained in terms of relatively long persistence at synapses of the excitatory changes produced by single volleys (Dale, 1937; Eccles, 1937; Barron and Matthews, 1938; Bronk, 1939). However, the unknown, but obviously complex, configuration of the tegmental projections from the hypothalamus allows

for the setting up of complicated chains of interneurones wherein synaptic changes of brief duration could account for the relations noted (Lorente de Nó, 1939).

SUMMARY

Stimulation of the hypothalamus of the anesthetized cat with brief repetitive condenser shocks of moderate intensity leads, after a latency of less than 0.1 second, to an abrupt increase in activity of sympathetic nerves to the heart and blood vessels. This activity ceases equally abruptly when stimulation is stopped, and for a variable period thereafter all spontaneous activity in these nerves is inhibited. There is no evidence under the conditions of our experiments that any sympathetic after discharge results from hypothalamic stimulation. Blood pressure begins to rise some 1 to 2 seconds after the start of hypothalamic stimulation, and may continue to rise and remain elevated several seconds after stimulation is stopped. The delay in the rise of blood pressure and the prolongation of the rise result from latency and inertia of the sympathetic effector, not from any corresponding delay or persistence of neural activity.

An increase in intensity or frequency of hypothalamic stimulation increases the magnitude and duration of the rise in blood pressure. This increased effector response is brought about by an increase in the number of sympathetic motor neurones set into activity and by an increase in the frequency of response of each neurone.

Multiple pathways descend from both sides of the hypothalamus to make connection with each sympathetic motor neurone. The frequency of response of the neurone is a function of the number of these pathways excited and of the frequency at which they are excited.

While stimulation of the lateral and posterior portions of the hypothalamus yields responses of greater magnitude, no qualitative differences have been noted on stimulation of the preoptic, tuberal or mammillary divisions.

The buffer reflexes which control the spontaneous sympathetic outflow from the medullary centers, also moderate the outflow induced by hypothalamic stimulation. Activation of the buffer afferents may inhibit all response of sympathetic motor neurones to hypothalamic stimulation or reduce the number of these neurones responding. Similarly the frequency of response of any single neurone may be reduced or the response entirely abolished by activation of the buffer afferents. The buffer afferents impress a pulse modulation upon mild increases of sympathetic activity which result from hypothalamic stimulation in exactly the same way that they modulate spontaneous sympathetic outflow. These facts are interpreted as indicating that sympathetic responses from hypothalamic stimulation are mediated through medullary sympathetic centers, not by direct connection of descending hypothalamic pathways with sympathetic motor neurones.

The frequency of firing of a sympathetic motor neurone in response to hypothalamic stimulation is determined by the level of excitation maintained by the hypothalamic volleys, the time course of the recovery cycle, and the degree of activity of inhibitory afferents at some critical point between hypothalamus and motor neurone.

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FORMATION OF THE R COMPLEX OF THE ELECTROCARDIOGRAM[1]

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In a previous study a method was described which permitted the separate registration of what appeared to be the contribution of the right and left ventricles to the electrocardiogram (1). It was concluded that the normal electrocardiogram is formed by the algebraic summation of these components, i.e., the dextro- and levocardiograms. The dextrocardiogram, a monophasic-like complex directed upward, was found to precede by a short interval the levocardiogram, a similar complex of opposite polarity. This asynchrony in excitation of the two ventricles has been noted before (2). A portion of the upstroke of the dextrocardiogram is therefore able to develop without opposition from the levocardiogram. This unopposed dextrocardiogram is recorded in the electrocardiogram as the upstroke of R.[3] The onset of the levocardiogram, which is of opposite polarity to the dextrocardiogram, arrests the further ascent of the dextrocardiogram and gives rise to the downstroke of R (fig. 1). The summit of the R complex therefore marks the moment when the major part of the surface of the left ventricle becomes electrically active, and the interval between the base and the summit of R (i.e., from the end of the downstroke of Q to the summit of R) represents the interval separating the onset of the dextro- and levocardiograms.

The amplitude of the R complex does not therefore necessarily indicate the height of the dextrocardiogram, since the levocardiogram may start before the full development of the dextrocardiogram. Certain evidence supports this view: 1, dextrocardiograms are seen occasionally which are higher than the R complex of the original electrocardiogram; 2, T waves

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[2] Fellow of the Dazian Foundation.

[3] The Q wave is not discussed for purposes of simplification. When Q is present in the electrocardiogram, the factors which are responsible for the R complex cannot be considered to be the primary events. The nature of Q and its relation to R will be considered in a later communication.

may be found with an amplitude exceeding R (3, fig. 2), and 3, the initial deflection of certain ventricular extrasystoles is *often greater than the* amplitude of the R wave in the *normal complex.*

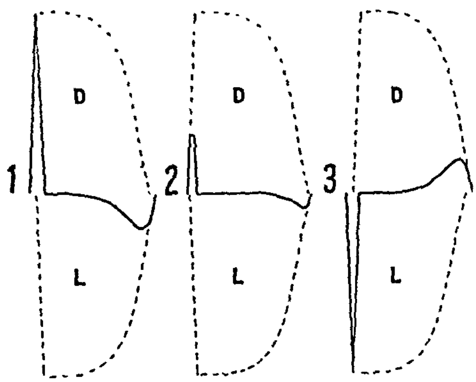

Fig. 1

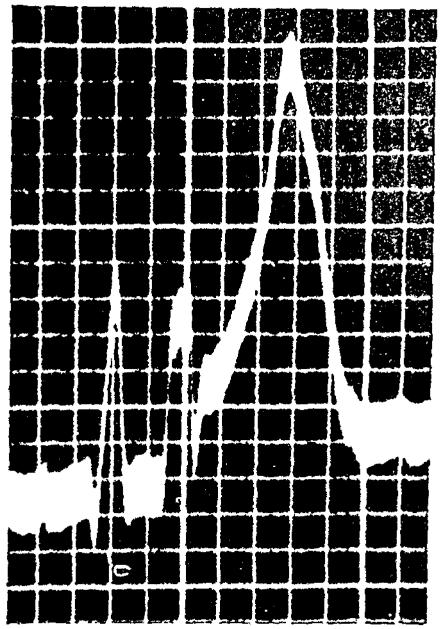

Fig. 1A

Fig. 1. A hypothetical diagram illustrating the formation of the R wave. In 1 the levocardiogram, *L*, begins at the instant the dextrocardiogram, *D*, reaches its full height. The resulting R complex (shown in heavy lines) is upright, and equals the dextrocardiogram in amplitude. In 2 the levocardiogram is initiated relatively sooner than in 1, and the R complex which results from this summation is lower, and its width is reduced. In 3 the levocardiogram precedes the dextrocardiogram and in this case the resulting R is directed downward.

It is obvious that in 2 a small plateau will develop at the apex of R if the dextro- and levocardiograms develop at equal velocities along a straight line. Such plateaus were in fact seen in several experiments (fig. 1a). In others (e.g., fig. 2c) no such plateau was seen despite extreme reduction in the amplitude of R. The absence of a plateau could be explained by postulating that the initial portions of the dextro- and levocardiograms do not develop at a constant velocity along straight lines, or that heating or cooling alters the velocity of development of the dextro- or levocardiogram.

Fig. 1a. Feb. 12, 1941. 10.5 kgm. Male dog. An enlarged photograph of a single complex taken from lead II after 12 minutes of heating the left ventricle at 50°C. This shows a reduction in the height of R and the plateau postulated in figure 1, no. 2.

Method. In the experiments reported in this paper, the hypothesis presented above concerning the formation of the R complex was tested by methods which presumably altered the interval separating the appearance of the dextro- and levocardiograms. Diminishing this interval should reduce the amplitude of R, while increasing the interval should augment R

by permitting the development of more of the upstroke of the dextrocardiogram before interference from the levocardiogram (fig. 1). Furthermore, if the levocardiogram should begin first, the initial deflection of the R complex should be downward. The amplitude of this complex should also depend upon the interval elapsing between the onset of the levocardio-

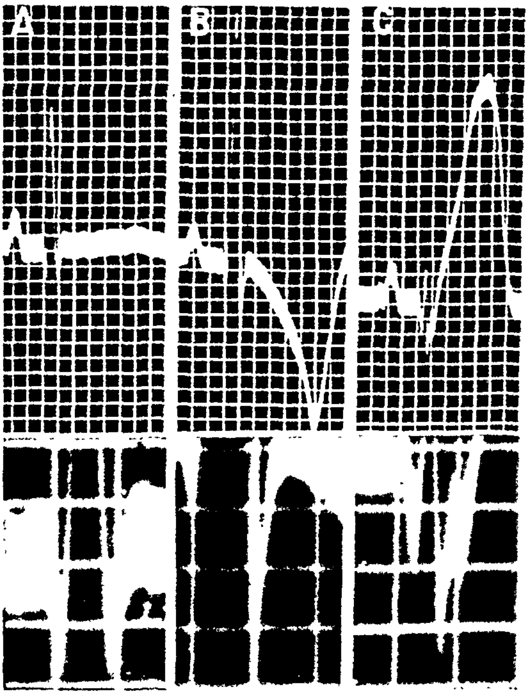

Fig. 2

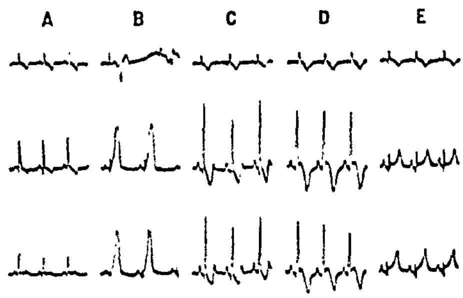

Fig. 3

Fig. 2. May 10, 1940. 7.5 kgm. dog. Enlarged photographs of electrocardiograms (lead III). (a) control, (b) after cooling the left ventricle, showing increased amplitude of R, and increase in the QR and QRS interval; (c) after heating the left ventricle, showing reduced amplitude of R, and shortening of the QR and Q-S interval. Below are greater enlargements of the Q-S interval.

Fig. 3. Nov. 24, 1940. 7.0 kgm. dog. Tracings of electrocardiograms which show the influence of heat and cold on the amplitude of the R complex. Tracings were employed because of the great amplitude of R in C and D and the consequent difficulty of adequate photographic reproduction. A. Control, in leads I, II, and III. B. After heating the left ventricle (50°C.). (Extrasystoles are seen in lead I.) C. Heating the right ventricle. D. Cooling the left ventricle (6°C.). E. Cooling the right ventricle.

gram and the onset of the dextrocardiogram. Two methods were employed. The first consisted in heating and cooling individual ventricles for several minutes in an endeavor to accelerate or delay the appearance of the excitatory process at the surface. The second method consisted in eliciting extrasystoles from symmetrically located points on the right and left ventricles equidistant from the septum. By this method the major portion of one or the other ventricle could be activated before the wave of

excitation reached the opposite ventricle. Bipolar electrodes, with an interpolar distance of 2 to 3 mm. were stitched to the epicardium and activated by a thyratron stimulator. Ten dogs were employed and were prepared as previously described (3).

RESULTS. A. *Effect on the R complex of heating and cooling individual ventricles.* Enlarged photographs of three typical R complexes are shown in figure 2; the first is the control complex (2A), the second (2B) one of increased amplitude produced by cooling the left ventricle, and the third (2C) a complex of low amplitude which followed warming the left ventricle. It can be seen that in the augmented R complex produced by cooling the left ventricle, the Q-R interval is increased, as might be expected if the onset of the levocardiogram were delayed. The Q-R (end of Q to apex of R) interval is decreased in the complex of low amplitude which was produced by heating the left ventricle. This is consistent with the supposition that the levocardiogram was initiated sooner. The influence of these alterations in Q-R upon the duration of the Q-S interval is shown in greater detail below the photographs of the whole complex.

The influence of thoroughly heating and cooling each ventricle on the amplitude of R is shown in figure 3. When the left ventricle was heated, R decreased in amplitude (3b) while its height increased when this ventricle was cooled (3d). Conversely, cooling the right ventricle diminished the height of R (3e) while warming increased it (3c). Consistent results were obtained in all experiments and could be reproduced as often as desired in each experiment. In some instances the increase above normal in the height of R was not striking, while the diminution was more readily obtained. The typical T wave changes produced by heat and cold consistently made their appearance as previously described (3, fig. 3). The changes in the R complex *a*, had a longer latency; *b*, required more time for their full development, and *c*, subsided more slowly after removal of the thermal chamber than the T wave changes which also appeared in response to heating or cooling. These differences in the evolution of changes in the R and the T complexes are summarized in figure 4.

B. *Direction of the R complex in extrasystoles from selected areas of right and left ventricles, equidistant from septum.* Figure 5 shows in confirmation of the theory presented in the introduction that extrasystoles elicited from the left ventricle exhibit a downward initial deflection in all three conventional leads (5b) while extrasystoles from the right ventricle show an initial deflection which is upward in all three leads (5a). One condition was essential to obtain such complexes, i.e., that the point of stimulation be sufficiently removed from the septum to permit spread of the impulse to the greater part of the ventricle stimulated before the opposite ventricle was involved. For this reason points were chosen approximately equidis-

tant from the septum. The configuration of extrasystoles elicited from points nearer to the septum is discussed in a following paper (4).

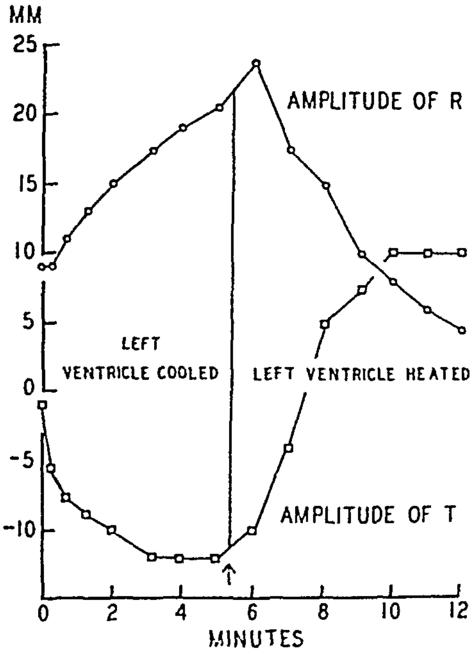

Fig. 4

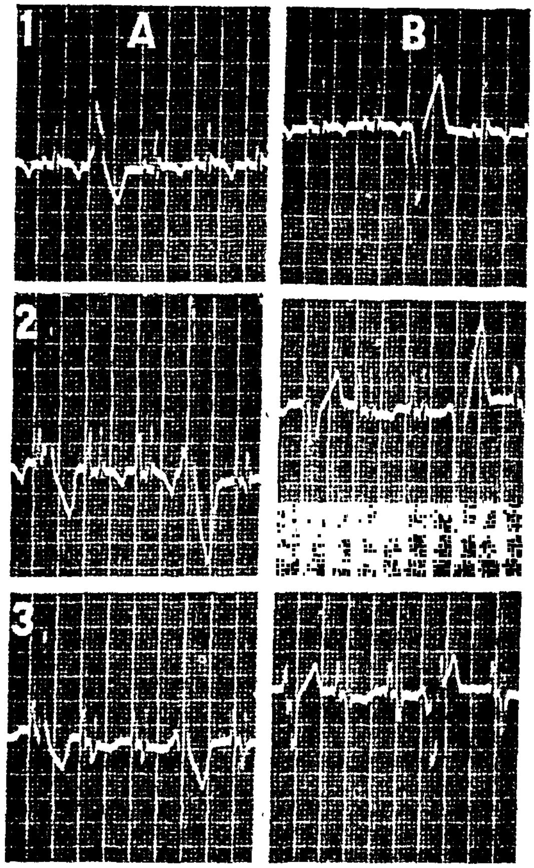

Fig. 5

Fig. 4. Same experiment as figure 1a showing the dissociation of R and T changes. In this figure are plotted the amplitude of R and T during cooling and then heating the left ventricle. Ordinates, amplitude of R and T in millimeters, abscissae, time in minutes. It is seen 1, that changes in the amplitude of T begin promptly when cooling starts while R remains for a time unchanged; 2, that the T wave reaches a maximum while the R wave continues to develop; 3, that the T wave responds promptly to the change from cooling to heating, while R continues to increase for a time before responding to cold; 4, that the T wave again levels off while R continues to change.

Fig. 5. Same experiment as figures 1a and 5. Electrocardiograms from the three leads, A showing extrasystoles elicited from the right ventricle, and B from the left ventricle. The points of stimulation were symmetrically placed on the right and left ventricles equidistant from the septum. The right ventricular extrasystole is initiated by an upright deflection in all three leads, while the left ventricular extrasystole begins with a downward initial deflection in all three leads.

Summary. 1. Heating the left ventricle or cooling the right ventricle for a sufficient time, shortened the Q-R interval and decreased the amplitude of R.

2. Heating the right ventricle or cooling the left ventricle lengthened the Q-R interval and increased the amplitude of R.

3. Extrasystoles elicited by primary activation of the major part of the left ventricle showed downward initial deflections in all three conventional leads.

4. Extrasystoles elicited by primary activation of the major part of the right ventricle exhibited an upright initial deflection in all three conventional leads.

CONCLUSIONS

1. The R complex of the electrocardiogram results from the algebraic summation of the initial portions of the dextro- and levocardiograms.

2. When the R complex is upright, its initial deflection is the upstroke of the dextrocardiogram, while the downstroke is produced by the onset and development of the levocardiogram.

3. When the R complex is directed downward, the downstroke is the initial portion of the levocardiogram, while the upstroke is produced by the onset and development of the dextrocardiogram.

4. The amplitude of the R complex varies with the interval separating onset of the dextro- and levocardiograms. The maximum amplitude is limited by the amplitude of the component dextro- or levocardiograms.

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THE NATURE OF LEADS I AND III OF THE ELECTROCARDIOGRAM[1]

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Findings reported in a foregoing paper permit the deduction that different areas of the heart are represented in leads I and III of the electrocardiogram (1). The reasoning upon which this deduction is based is as follows:

When the electrical activity of the anterior surface of the heart is abolished by a potassium pledget covering portions of both ventricles, a dextrocardiogram is recorded in lead I and a levocardiogram in lead III. Lead II may show a practically normal complex. With such treatment of the anterior surfaces, only the posterior surfaces of the ventricles can be the source of action potentials; therefore, the dextrocardiogram in lead I must be derived from the posterior surface of the right ventricle (posterior dextrocardiogram). The presence of a levocardiogram in lead III indicates similarly that lead III has recorded from the posterior surface of the left ventricle (posterior levocardiogram). When the posterior surfaces of the ventricles are treated with potassium to abolish action potentials from this region, a levocardiogram is obtained in lead I, while a dextrocardiogram is found in lead III. Lead II again may show only minor deviations from the control, provided that equal surfaces of the right and left ventricles are treated. In this case the levocardiogram in lead I must be recorded from the anterior surface of the left ventricle (anterior levocardiogram), while the dextrocardiogram in lead III must be derived from the anterior surface of the right ventricle (anterior dextrocardiogram).

The inference may therefore be drawn that 1, contiguous regions of the right and left ventricles do not participate in the interference which produces the electrocardiogram in leads I and III; 2, lead I records, at least preponderantly, the interference between the action potentials of the anterior surface of the left ventricle, and the posterior surface of the right ventricle (i.e., lead I = posterior dextrocardiogram + anterior levocardio-

[1] Aided by grants from Fluid Research Funds, Yale University and Emanuel Libman Fellowship Fund.

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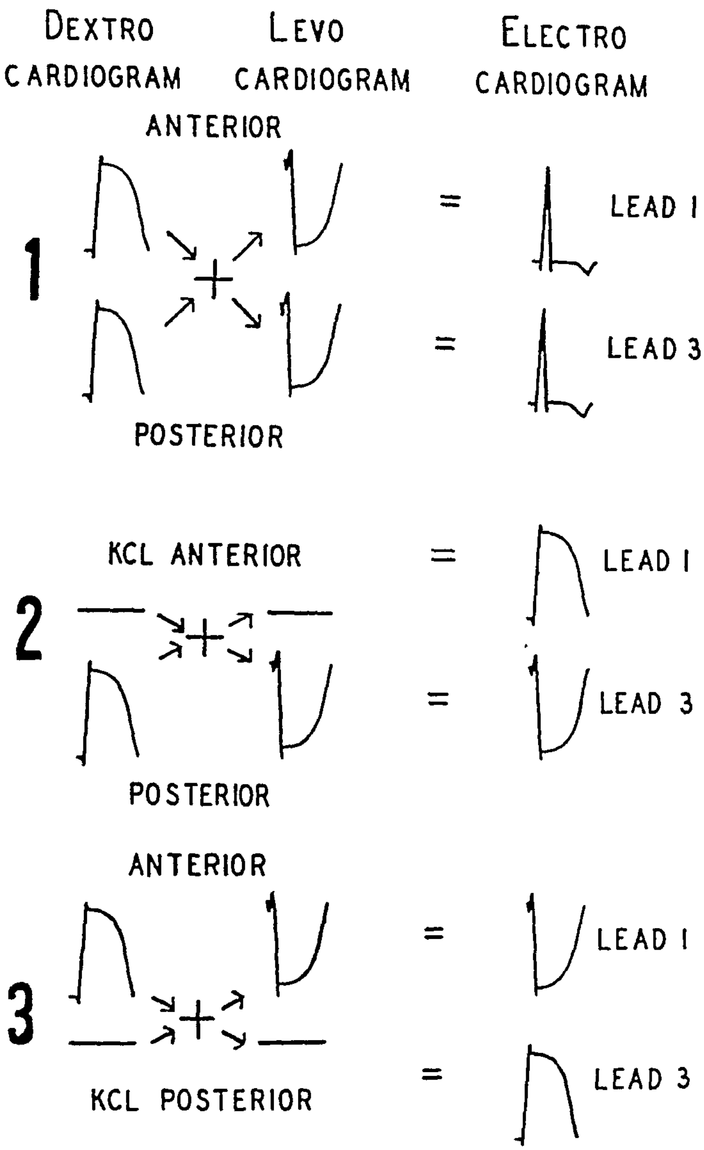

Fig. 1. A diagram illustrating in section 1 the hypothesis that lead I is formed by the algebraic summation of the anterior levocardiogram and the posterior dextrocardiogram, while lead III is formed by the summation of the anterior dextrocardiogram and the posterior levocardiogram. In this nomenclature it is assumed that each ventricle can be divided into an anterior and posterior region. Dextro- and levocardiograms are drawn as they are most frequently recorded. A Q wave is seen in the dextrocardiogram and a Q and small R are seen in the levocardiogram. These waves are included because they indicate the slightly earlier onset of the main initial deflection of the dextrocardiogram compared with the levocardiogram. They are not to be interpreted as an integral component of either the dextro- or levocardiogram. The diagram in section 2 explains the effect of abolishing action potentials from the anterior surfaces of both ventricles by the application of KCl. The diagram in section 3 explains the effect of abolishing the posterior dextro- and levocardiograms by KCl.

gram); 3, lead III records the interference between the action potentials of the anterior surface of the right ventricle and the posterior surface of the left ventricle (lead III = anterior dextrocardiogram + posterior levocardiogram); 4, lead II appears to record from the entire heart, being the summation of effects in leads I and III. Figure 1 illustrates these conclusions. In the following experiments further evidence is developed in support of the theory that leads I and III record from different surface areas of the heart as described above in 2 and 3.

A. *Leads in which T wave changes appear when specific areas of the surface of the heart are heated and cooled.* Heat and cold are known to shorten and lengthen, respectively, the dextro- or levocardiogram, thereby producing characteristic T wave changes (2). When heat or cold is applied to the surface of the heart the characteristic T wave changes which result serve to identify the ventricle so treated. In the following experiments, contiguous areas of both the right and left ventricles were treated simultaneously, to determine the nature of the T wave changes in leads I and III. In other experiments portions of the surface of a single ventricle were heated and cooled to determine the lead in which the resulting T wave changes appeared. A third procedure consisted in producing T wave changes in a single lead by thermal application to a chosen area of one ventricle and then exploring the opposite ventricle with a second thermal chamber to find the region which interferes with the effects of the first application. Ten dogs were employed, prepared as described previously (2).

Results of heating and cooling selected areas of the surface of the heart. When the anterior surface of the heart was cooled by a thermal chamber covering portions of both right and left ventricles, oppositely directed T waves appeared as follows: *a*, a prolonged, inverted T wave appeared in lead I, indicating in this lead the influence of a prolonged levocardiogram which could have been derived only from the cooled anterior surface of the left ventricle; *b*, in lead III the T wave was upright and prolonged, indicating the influence in this lead of a prolonged dextrocardiogram which could have been derived only from the anterior surface of the right ventricle (fig. 2 C). Lead I therefore must have recorded preponderantly from the anterior surface of the left ventricle, while lead III must have recorded from the anterior surface of the right ventricle (fig. 3, section 1). The results obtained by warming the anterior surface also showed oppositely directed T waves in leads I and III (fig. 2 B). In this case, however, T_I was upright, indicating shortening of the anterior levocardiogram, while T_{III} was inverted, indicating curtailment of the anterior dextrocardiogram. This substantiates the hypothesis that the anterior levocardiogram is recorded in lead I, while the anterior dextrocardiogram is recorded in lead III (fig. 3, section 3).

Warming the posterior surfaces of both ventricles produced an inverted

T wave in lead I and an upright T wave in lead III (fig. 2 E). The inverted T wave in lead I indicates the existence of a shortened dextrocardiogram which could have been derived only from the warmed posterior surface of the right ventricle. The upright T wave in lead III indicates a shortening of the posterior levocardiogram, which could have been derived only from the warmed posterior surface of the left ventricle (fig. 3, section 4).

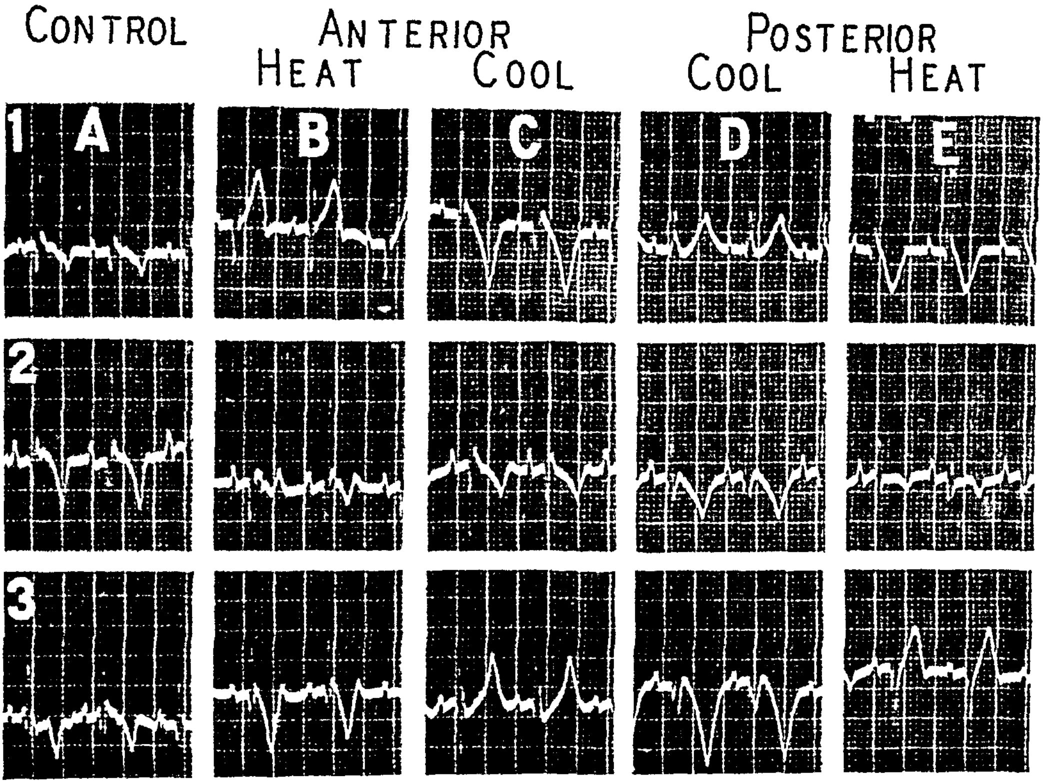

Fig. 2. Dog. Feb. 7, 1941. A. Control, leads I, II, and III. B. The influence of heating the anterior surfaces of the right and left ventricles (50°C.). T_I is sharply upright and T_{III} sharply inverted. Q-T duration unchanged. C. Cooling (5°C.) the anterior surfaces of both ventricles. T_I sharply inverted, T_{III} sharply upright. Q-T interval prolonged. D and E, comparable records obtained after cooling and heating the posterior surfaces of both ventricles.

Cooling the posterior surfaces of both ventricles produced an upright T_I and an inverted T_{III} (fig. 2 D). The upright T_I indicates the presence of a prolonged dextrocardiogram, which could have been derived only from the cooled posterior surface of the right ventricle. The inverted T_{III} similarly indicates the presence of a prolonged levocardiogram, which must have been derived from the cooled posterior left ventricle (fig. 3, section 2).

These experiments, which are summarized in figure 3, indicate that lead I records predominantly from the anterior surface of the left ventricle, and the posterior surface of the right ventricle (anterior levocardiogram

and posterior dextrocardiogram) whereas in lead III is recorded preponderantly the electrical activity of the anterior surface of the right ventricle, and the posterior surface of the left ventricle (anterior dextrocardiogram and posterior levocardiogram).

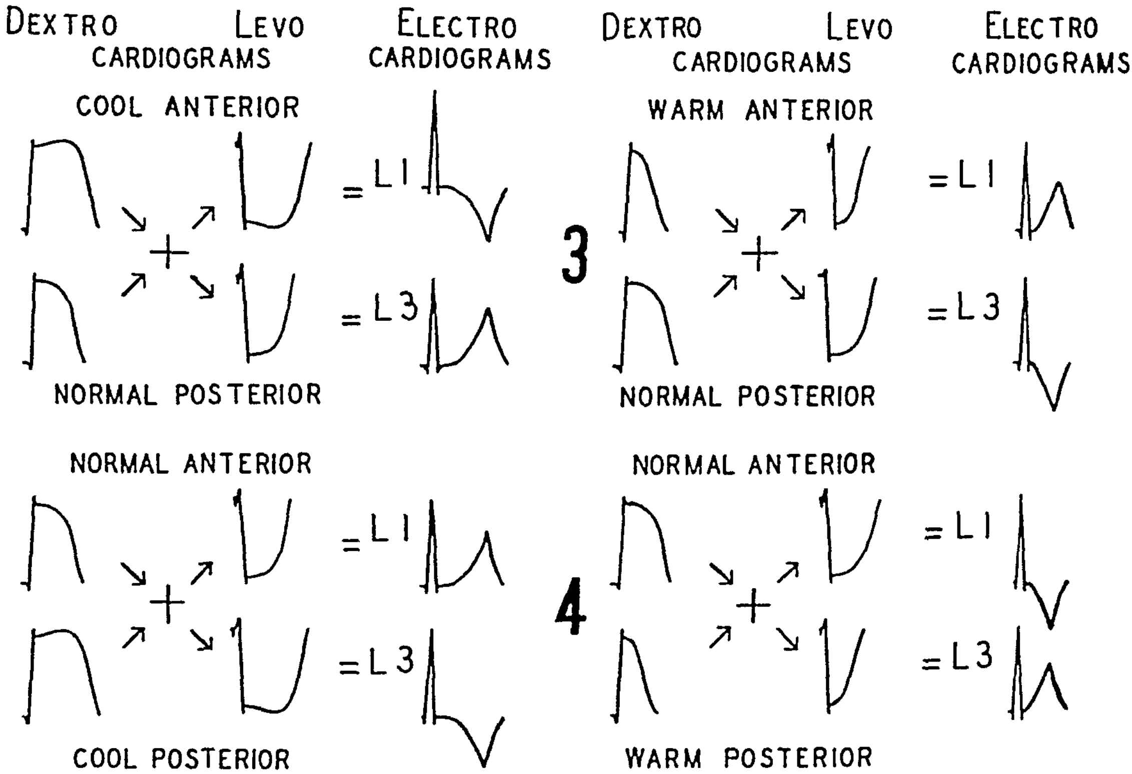

Fig. 3. A diagram illustrating the mechanism of changes produced in the electrocardiogram by heating and cooling the surface of the heart, to be compared with actual records in figure 3. In section 1 the anterior surface is cooled, prolonging the anterior dextro- and levocardiograms. Summation as proposed in the theory developed in this paper produces an inverted T_I and an upright T_{III}. The Q-T interval is prolonged in both leads. Warming the same region (section 3) shortens the anterior dextro- and levocardiograms, and results in an upright T_I and an inverted T_{III}. The duration of Q-T is unchanged in both leads. In sections 2 and 4 the influence of cooling and warming the posterior surfaces of both ventricles is illustrated. For the purpose of simplification, the time of onset of the dextro- and levocardiograms is not changed in the diagrams. In actual experiments, heating or cooling does eventually alter the time of onset of the dextro- and levocardiograms, and produces changes in the amplitude of the R complex (see figs. 4 and 5).

A further test of the validity of the hypothesis presented here consisted in producing characteristic alterations in the T wave by warming or cooling the anterior or posterior surface of a single ventricle to determine *a*. in which lead the effect was manifest, and *b*, where on the other ventricle a similar simultaneous thermal application would neutralize the first alterations. It was found that lead I recorded changes due to heating and

cooling the anterior left and posterior right ventricular surfaces while lead III reflected events at the anterior right and posterior left ventricular surfaces.

The effects of applications of heat or cold to the anterior surface of the right ventricle, which appear predominantly in lead III, were neutralized by a similar application to the posterior surface of the left ventricle. Likewise, heating or cooling the anterior surface of the left ventricle interfered with the effects of similar thermal changes at the posterior surface of the right ventricle. These changes appeared predominantly in lead I, but completely satisfactory records of this interference were not obtained, due to difficulty in placing and maintaining the thermal chamber in a proper position on the posterior surface of the right ventricle.

B. *Alterations in the R complex of leads I and III produced by heating and cooling anterior and posterior surfaces of both ventricles.* The R complex results from the algebraic summation of the initial portions of the dextro- and levocardiograms (3). The upstroke to the peak of R is formed by the action current of the right ventricle, while the downstroke is developed by the onset of activity in the left ventricle. The height of R is therefore in part determined by the interval between the activation of the two ventricles (3). If this interval is shortened, the amplitude of R must decrease, while if it is lengthened, the amplitude should increase (3). Application of cold or heat to a ventricle will, after a sufficient interval, alter the arrival of the cardiac impulse at the surface of the region treated, delaying or hastening its activation in relation to the other ventricle, and thus produce characteristic alterations in the amplitude of R (3). When, then, areas of both ventricles are simultaneously heated or cooled, the resulting changes in R will indicate which ventricle is responsible for them.

METHOD. Heating and cooling of the anterior and posterior surface of the heart involving both ventricles was carried out in 7 experiments as described previously. The resulting changes in the height of R were determined.

RESULTS. Figures 4 and 5 illustrate the influence on the height of the R complex in leads I and III of heating and cooling anterior and posterior septal regions. Heating the anterior surface reduced the amplitude of R_{I} and increased R_{III} (fig. 4 C) while cooling had the opposite effect (fig. 4 B). Conversely, heating the posterior surface increased the amplitude of R_{I} and reduced R_{III} (fig. 5 C); cooling decreased R_{I} and increased R_{III} (fig. 5 B). The significance of these results is discussed in the following paragraphs.

When the posterior surfaces of both ventricles were cooled the amplitude of R_{I} diminished while the amplitude of R_{III} increased. Cooling necessarily delayed the arrival of the impulse to the two posterior surfaces and produced a delayed posterior dextrocardiogram and a delayed posterior

levocardiogram. The influence of the delayed posterior dextrocardiogram was found in lead I in the reduced amplitude of R. This indicates that the posterior dextrocardiogram is recorded selectively in lead I. The influence of the delayed posterior levocardiogram was found in the increased amplitude of R in the lead III. The posterior levocardiogram is therefore shown to appear selectively in lead III.

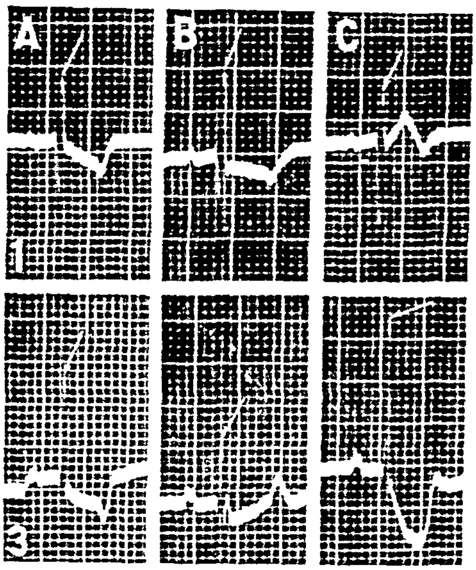

Fig. 4

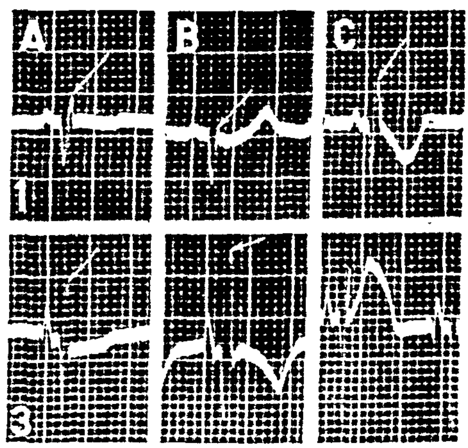

Fig. 5

Fig. 4. Dog. 10 kgm. April 25, 1941. Leads I and III. Arrows point to apex of R complex. Unretouched photographic enlargements.

A. Control with thermal chambers at body temperature in place on anterior surfaces of right and left ventricle.

B. Cooling (0°C.). Increased R_I, diminished R_{III}.

C. Warming (48°C.). Marked reduction of R_I, increase in R_{III}.

Fig. 5. Dog. 11.3 kgm. April 23, 1941. Leads I and III. Arrows point to apex of R complex. Unretouched photographic enlargements.

A. Control. Thermal chambers at body temperature in place on posterior surfaces of the right and left ventricles.

B. Cooling (0°C.). Reduction in R_I and increase in R_{III}.

C. Heating (48°C.). R_I increased, R_{III} diminished.

Heating the posterior surfaces of both ventricles produced an augmented R_I and diminished R_{III}. Warming these regions hastened the initiation of the posterior dextro- and levocardiograms. The influence of the premature onset of the posterior dextrocardiogram was shown in the increased amplitude of R_I, indicating that the posterior dextrocardiogram is recorded selectively in lead I. The influence of the premature onset of the posterior levocardiogram was shown in the decreased amplitude of R_{III}, indicating that the posterior levocardiogram is recorded selectively in lead III.

Similar reasoning applied to the influence of heating and cooling the anterior surface of both ventricles on the amplitude of R_I and R_{III} permits the deduction that the anterior dextrocardiogram is recorded selectively in lead III and the anterior levocardiogram is recorded selectively in lead I.

In these experiments, as in those reported in the previous section, lead II recorded the approximate algebraic summation of lead I and lead III.

SUMMARY. 1. Cooling the anterior surfaces of both right and left ventricles produced an inverted prolonged T_I and a prolonged upright T_{III}. R_I was increased and R_{III} was diminished in amplitude.

2. Cooling the posterior surfaces of both right and left ventricles produced a prolonged upright T_I and a prolonged inverted T_{III}. R_I was diminished and R_{III} augmented.

3. Heating the anterior surfaces of both right and left ventricles produced an upright T_I of normal duration and an inverted T_{III} of normal duration. R_I was decreased in amplitude and R_{III} was increased.

4. Warming the posterior surfaces of both right and left ventricles produced an inverted T_I of normal duration and an upright T_{III} of normal duration. R_I was increased and R_{III} decreased in amplitude.

CONCLUSIONS

1. Lead I records the algebraic summation of the anterior levocardiogram and the posterior dextrocardiogram.

2. Lead III records the algebraic summation of the anterior dextrocardiogram and the posterior levocardiogram.

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CONFIGURATION OF ANTERIOR AND POSTERIOR SEPTAL EXTRASYSTOLES IN THE STANDARD LEADS OF THE ELECTROCARDIOGRAM[1]

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In the experiments to be reported here, a study was made of the configuration of extrasystoles elicited from the septum and immediately adjacent regions. It would be expected that the radial spread of an impulse starting at or near the septum would produce simultaneous excitation of adjacent areas of both right and left ventricles. Thus, an impulse originating at the anterior septum midway between apex and base would be expected to excite the anterior portions of the right and left ventricle before it reached the posterior surfaces of the heart. Conversely, a stimulus applied to the posterior septum would be expected to evoke simultaneous response in the posterior surfaces of the right and left ventricle before the anterior surfaces were activated.

Evidence has already been presented (1) that lead I records the interference between the anterior levocardiogram and the posterior dextrocardiogram. Lead III records a similar interference between the posterior levocardiogram and the anterior dextrocardiogram. According to this formula, extrasystoles originating in the anterior or posterior septal regions should exhibit predictable configurations. When an extrasystole is elicited by stimulation of the anterior septum, thus exciting simultaneously the anterior surfaces of the right and left ventricles, the anterior levo- and dextrocardiograms should both begin before their posterior counterparts. Therefore, the first electrical activity of these extrasystoles recorded in lead I should be the unopposed downstroke of the anterior levocardiogram. Similarly, the first electrical activity of the extrasystole appearing in lead III should be the unopposed upstroke of the anterior dextrocardiogram (fig. 1A).

By similar reasoning it may be predicted that posterior septal extrasystoles should show an upward initial deflection in lead I derived from

[1] Aided by grants from Fluid Research Funds, Yale University and Emanuel Libman Fellowship Fund.

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the unopposed upstroke of the posterior dextrocardiogram and a downward deflection in lead III derived from the unopposed initial downward deflection of the posterior levocardiogram (fig. 1B).

METHOD. Six dogs were employed. They were prepared as described previously (1). Extrasystoles were elicited by delivering periodic break

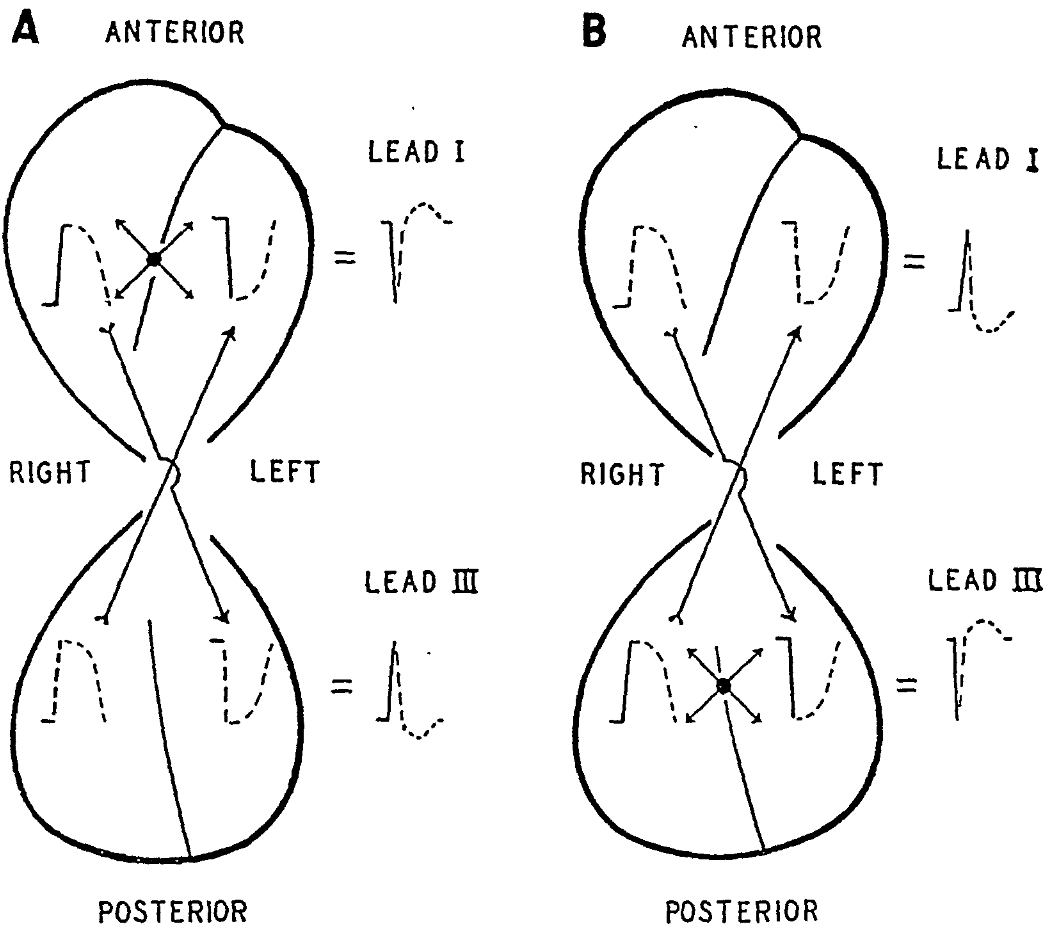

Fig. 1. A diagram illustrating the configuration of anterior and posterior septal extrasystoles. An extrasystole originating at the anterior septum (A) will involve the anterior surfaces of both right and left ventricles before spreading to the posterior surfaces, as is indicated by the heavy lines of the anterior dextro- and levocardiograms. Since the anterior levocardiogram is recorded in lead I, the initial deflection in this lead will be downward. The anterior dextrocardiogram is recorded in lead III, producing an initial upward deflection in this lead. A similar explanation for the configuration of posterior septal extrasystoles is presented in B.

shocks from a thyratron stimulator through bipolar electrodes stitched to the epicardium.

RESULTS. Figure 2 shows the configuration of extrasystoles in the three leads when the anterior surfaces of both ventricles were activated first (C), and when the initial activation involved the posterior surfaces of

both ventricles (D). As predicted above, the initial deflection of the ectopic beats from the anterior septum was downward in lead I and upward in lead III. The initial deflection of extrasystoles evoked by stimulation of the posterior septum was directed upward in lead I and downward in lead III. Consistent with other evidence (3) is the finding that extra-

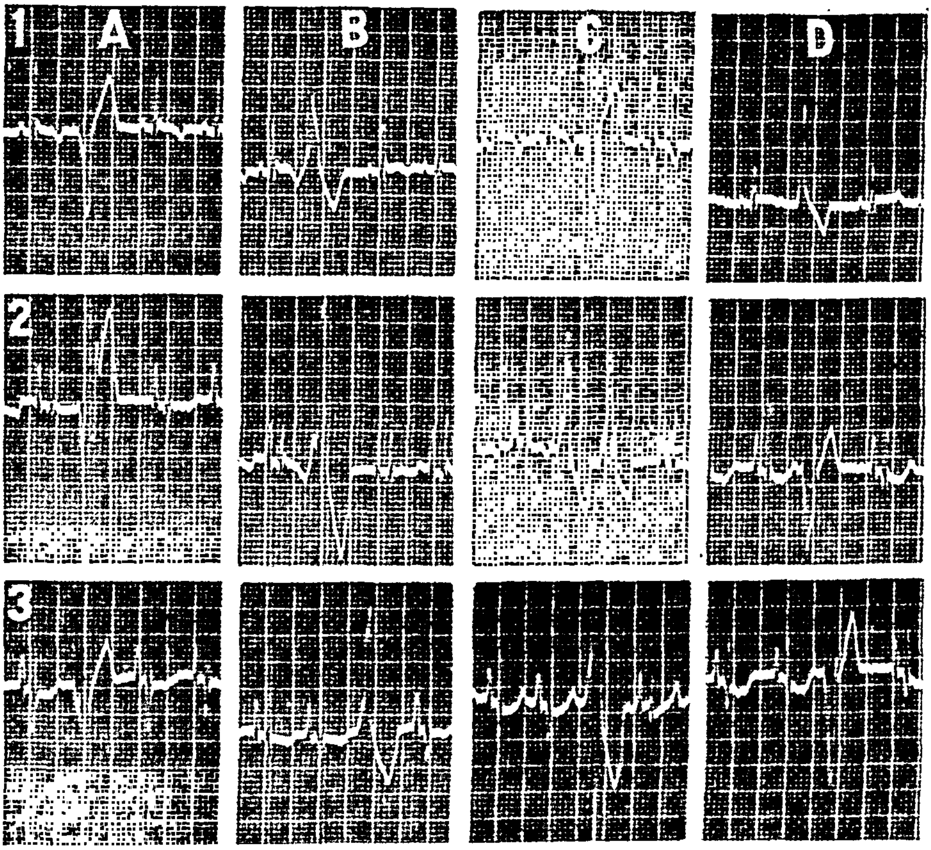

Fig. 2. Dog. Feb. 12, 1941. A, leads 1, 2, 3, showing extrasystoles elicited by bipolar stimulation near the center of the surface of the left ventricle. Initial deflections downward in all three leads. B, extrasystoles from the center of the right ventricular surface, showing an upright initial deflection in all three leads. C, extrasystoles elicited from the mid-point of the anterior septum. A downward initial deflection in lead I and an upright initial deflection in lead III. D, extrasystoles elicited from the posterior septum. Upright initial deflection in lead I and downward initial deflection in lead III.

systoles from the septum at the apex have the same configuration as extrasystoles from the posterior septum.

Extrasystoles elicited from regions lying between the lateral areas, which gave complexes with the same direction in all three leads, and the septal areas giving the oppositely directed complexes in leads I and III as described above, showed transitional patterns which will be discussed in another communication.

Discussion. The configuration of extrasystoles obtained in these experiments is consistent with the theory that distinct regions of the heart are selectively represented in leads I and III; i.e., lead I records the algebraic summation of the anterior levocardiogram and the posterior dextrocardiogram, while lead III records a similar summation of the anterior dextrocardiogram and the posterior levocardiogram.

These experiments, together with those reported previously (2), permit certain statements concerning the origin of ventricular extrasystoles. A downward initial deflection in lead I indicates that the anterior surface of the left ventricle was excited before the posterior surface of the right ventricle, while an upward initial deflection in this lead indicates that the posterior surface of the right ventricle was excited before the anterior surface of the left ventricle. A downward initial deflection in lead III indicates that the posterior surface of the left ventricle was excited before the anterior surface of the right ventricle, while an upward initial deflection in this lead indicates that the anterior surface of the right ventricle was excited before the posterior surface of the left ventricle.

An upward initial deflection in both leads I and III indicates that the major portion of the right ventricle was excited in advance of the left ventricle. Conversely, a downward initial deflection in both leads I and III indicates that the major portion of the left ventricle was excited before the right ventricle. Such configurations arise when extrasystoles originated near the center of the ventricular surface (2).

When the initial deflections of an extrasystole are oppositely directed in leads I and III, either the anterior or posterior surfaces of both ventricles must have been excited more or less simultaneously, and in advance of the opposite surfaces. Extrasystoles originating at or near the anterior septum exhibit a downward initial deflection in lead I and an upward initial deflection in lead III. Extrasystoles originating at or near the posterior septum show an upward initial deflection in lead I and a downward initial deflection in lead III.

Electrocardiograms showing in lead I an upward QRS and in lead III a downward initial deflection are now said to indicate "left ventricular preponderance," or "left axis deviation." A downward initial deflection in lead I and an upward initial deflection in lead III are termed "right ventricular preponderance," or "right axis deviation." It can be seen from the experiments reported above that when the anterior surfaces of both ventricles are activated before the posterior surfaces, the direction of the initial deflection in leads I and III is what would be interpreted as "right axis deviation." Conversely, primary activation of the posterior surfaces of both ventricles gives rise to configurations which are interpreted as "left axis deviation."

It is also interesting to compare the patterns of extrasystoles from the

anterior or posterior septum with electrocardiograms interpreted as indicating right or left bundle-branch block. The pattern of upward initial deflection in lead I and a downward initial deflection in lead III, characteristic of left bundle-branch block, is also characteristic of extrasystoles produced by primary activation at the posterior surface, while a downward initial deflection in lead I and an upward deflection in lead III, characteristic of right bundle-branch block, would in an extrasystole signify the primary activation of the anterior surfaces of both ventricles.

SUMMARY

1. Extrasystoles elicited from the anterior septum show a downward initial deflection in lead I and an upward initial deflection in lead III.

2. Extrasystoles elicited from the posterior septum and apex show an upward initial deflection in lead I and a downward initial deflection in lead III.

3. These findings support the theory that lead I records the algebraic summation of the anterior levocardiogram and the posterior dextrocardiogram, while lead III records the summation of the anterior dextrocardiogram and the posterior levocardiogram.

4. Extrasystoles from the anterior septum are comparable with ventricular complexes at present interpreted as having "right axis deviation" or "right bundle-branch block."

5. Extrasystoles from the posterior septum are comparable with ventricular complexes interpreted as showing "left axis deviation" or "left bundle-branch block."

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THE VERTICAL BALLISTOCARDIOGRAPH; EXPERIMENTS ON THE CHANGES IN THE CIRCULATION ON ARISING; WITH A FURTHER STUDY OF BALLISTIC THEORY

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Progress in the physiological aspects of our work with the ballistocardiograph (Starr, Rawson, Schroeder and Joseph, 1939) has taken three directions. Theoretical studies on the origin of the impacts have been continued, leading to a change in certain of our views and the clarification of others.

A second ballistocardiograph has been constructed to obtain records in the standing or sitting position.

By this means we have studied the changes occurring in the cardiac output immediately after assuming the erect position, a time when some normal persons and many patients exhibit transient symptoms of dizziness or lightheadedness. Comparisons of the cardiac output lying and standing have been made by many students of the subject, but the results obtained have differed. This literature has been reviewed by McDowell (1938). We have made over 100 estimations on 58 normal subjects. Our results show that the average cardiac output per minute remains the same after assuming the erect posture, although some individuals consistently have smaller cardiac outputs per minute standing than lying. When the circulation in reclining subjects is above average normal it tends to diminish when the subject stands; when below average normal, it tends to remain the same or to rise. This same tendency can be demonstrated in many of the published results and it will explain some of the discrepancies found.

We have also studied methods of changing the relationship between the amount of the circulation in the two positions. This could be greatly altered by the drug paredrine, and also by the application of an abdominal binder in certain subjects.

Our theoretical studies were much assisted by Dr. LeRoy Williams who made two casts of the ventricular chambers for us and permitted us to make measurements in the dissecting room. We are also indebted to Dr. Hugo Roesler for advice concerning the position of the axes of the cardiac chambers.

STUDIES ON BALLISTIC THEORY. *A mechanical analogy.* Most readers will recall what happens when a long freight train, stopped on the track, is started by the locomotive. The train is not set in motion as a unit, but car by car, the impulse traveling down the train so that the last car moves some time after the first has started. Bend such a train around a curve like the aortic arch, have the locomotive push rather than pull, have it start, move a short distance and stop again, assume a second train for the pulmonary system and one has a reasonably close analogy to what happens in the circulation. The impacts of such a system will be the sum of the impacts of its units (cars) and these will be delivered in various directions due to the curve of the track and at different times as each starts and stops a little later in time than the one before it.

We propose to analyze the ballistics of the circulation according to this analogy by dividing it into units, analogous to the cars, the impulse traveling down the train as it starts being analogous to the pulse wave velocity. Thus we will estimate the impacts which would arise from the moving blood of subject Sta. during a single systole. This subject was chosen, not only for convenience, but also because he had proved to be an average subject in our previous investigation (Starr et al., 1939).

Construction of a schematic aorta. A diagram of the aorta of Sta. (fig. 1, A) was constructed from expectations based on Bazett's data, the relative measurements being influenced by those obtained from a cadaver of similar age found in the dissecting room. Great exactitude was not believed possible.

The section area of the schema's ascending aorta before the branches was placed at 3.64 sq. cm., the size expected from Bazett's data. The volume of the ascending aorta and the arch is 64 cc. while the nearest corresponding figure in Bazett's data, which includes the volume of the branches for some distance, is 81 cc. The volume of the schema's thoracic and abdominal aorta to the bifurcation is 80 cc. which can be compared with 101 cc. in Bazett's data, the latter figure including the femorals and the mouths of the larger branches.

In figure 1, A we have sought to divide this schematic aorta into cylindrical segments the contents of which would move to the segment next beyond whenever 10 cc. left the heart. Having no branches, the ascending aorta has been divided into segments of 10 cc. each. When the branches are reached, a part of the blood leaves the aorta and we have assumed that the blood thus diverted to the segmental vessels was proportional to the diminution in size of the aorta found in the cadaver, and continued this conception to the bifurcation. The volumes of segments used in the calculation but not shown in figure 1, A, from A XI to A XX, are: 5.6, 5.3, 5.0, 4.8, 4.4, 4.1, 3.9, 3.6, 3.3, and 3.1 cc.

A schema of the pulmonary artery divided into similar segments is also given in figure 1, B.

Relations of space and time. Thus in figure 1, A one can visualize the positions which the various units of blood will occupy *in space* as the heart contracts. Equally important is the *time* at which the blood units reach the successive positions. To obtain the latter, the area under Machella's (1936) blood velocity curve (fig. 1, C) was estimated by drawing it on cross section paper and counting the squares, and this area was divided by vertical lines into 6 equal parts (fig. 1, C). The intersections of these dividing lines with the abscissa are labeled Instant 1, 2, etc., and they indicate the times at which a unit of blood reaches the successive positions. Thus the blood occupying the proximal position in the aorta (A I, fig. 1, A) before systole begins at Instant I in time, will occupy, in succession, position A II in space at Instant 2 in time, A III at Instant 3, etc.

Calculation of impacts from blood in the great vessels. To calculate the impact of any blood unit in the aorta, we need first to determine its longitudinal velocity, next its longitudinal acceleration, and then to multiply the ordinates of the acceleration curve by the mass concerned. The method used for all the units can be illustrated by calculating the impacts arising from the blood in the proximal aorta, Unit A I, in its course around the aortic arch during a single systole.

In traveling from A I to A III in space and from Instant 1 to 3 in time, the course of Unit A I is longitudinal so Machella's (1936) curve is followed to Instant 3 in time. When traveling around the aortic arch, the longitudinal velocity diverges markedly from the true velocity and the former has been calculated by multiplying the ordinates of the true velocity curve by the cosines of the successive angles of divergence from the longitudinal. Angle Θ (fig. 1, A) which corresponds to this angle of divergence can be used more conveniently. The result is shown by the broken line in figure 1, C.

The next step is to calculate the longitudinal acceleration by differentiating the longitudinal velocity curve; the curve resulting is shown in figure 1, D.

We may now calculate the force of the impacts by multiplying the differential curve by the mass involved. The specific gravity of the blood being approximately 1, this remains 10 grams (1 mass unit) until the blood passes from position A III to A IV (fig. 1, A) during which change part of the blood is lost to the large branches, the mass remaining in the aorta diminishing as a result. Accordingly, to estimate the force of the impact we have multiplied the curve by a mass factor of 1 until the position A IV is attained where it is multiplied by 0.91. At A V, it is multiplied by 0.71, at A VI and VII by 0.67. Therefore the impact curve diverges from the acceleration curve as is shown by the dotted line in figure 1, D.

This method was employed for each of the segments in the aorta down to its bifurcation and for the pulmonary artery as well. Typical representatives of the family of impact curves resulting are shown in figure 1, F.

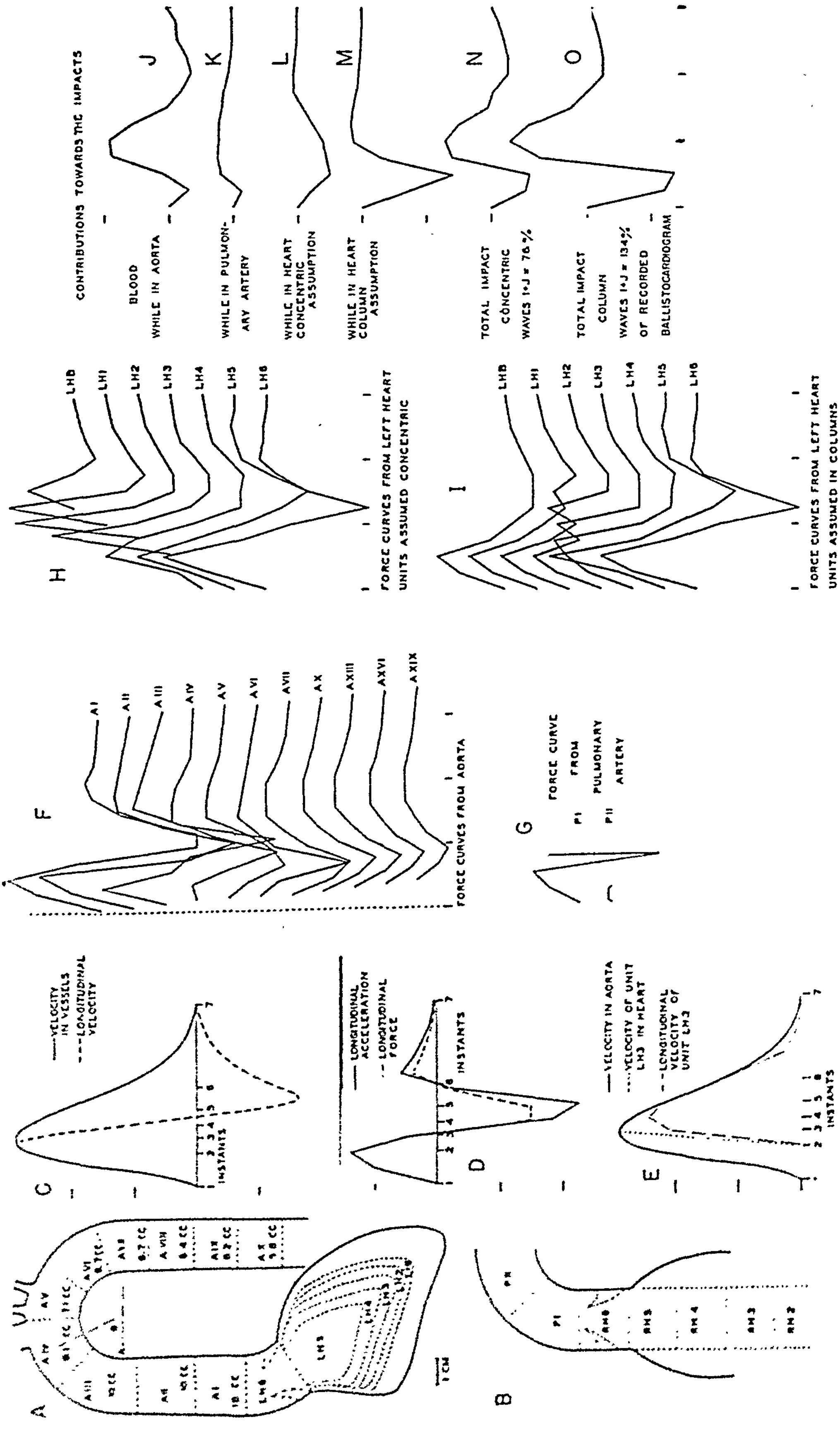
A
B
1 CM
C
VELOCITY IN VESSELS
LONGITUDINAL VELOCITY
INSTANTS
D
LONGITUDINAL ACCELERATION
LONGITUDINAL FORCE
INSTANTS
E
VELOCITY IN AORTA
VELOCITY OF UNIT LH3 IN HEART
LONGITUDINAL VELOCITY OF UNIT LH3
INSTANTS
F
AI
AII
AIII
AIV
AV
AVI
AVII
AX
AXIII
AXVI
AXIX
FORCE CURVES FROM AORTA
G
FORCE CURVE FROM PULMONARY ARTERY
PI
PII
H
LHB
LH1
LH2
LH3
LH4
LH5
LH6
FORCE CURVES FROM LEFT HEART UNITS ASSUMED CONCENTRIC
I
FORCE CURVES FROM LEFT HEART UNITS ASSUMED IN COLUMNS
CONTRIBUTIONS TOWARDS THE IMPACTS
BLOOD WHILE IN AORTA
J
WHILE IN PULMONARY ARTERY
K
WHILE IN HEART CONCENTRIC ASSUMPTION
L
WHILE IN HEART COLUMN ASSUMPTION
M
TOTAL IMPACT CONCENTRIC WAVES I+J = 76%
N
TOTAL IMPACT COLUMN WAVES I+J = 134% OF RECORDED BALLISTOCARDIOGRAM
O

Fig. 1

The final problem is to place these curves with relation to time. The units of blood in the aorta move in a sequence determined by the pulse wave velocity. To estimate this for any unit we have measured its distance from the aortic or pulmonary valves and then used average figures for pulse wave velocity given by Bazett (1940) for subjects of middle age, i.e., 3.3 meters per second, the heart to subclavian pulse wave velocity, for the ascending aorta, the transverse aorta, and the pulmonary artery; 7.6 meters per second, the subclavian to femoral velocity, for the descending aorta.

Calculation of impacts from blood in the heart. The position of the main axes, the lines from the centers of mass of the ventricular blood to the center of the outlet valve rings is clearly shown by Roesler's (1940) illustrations comparing x-ray pictures of hearts taken during life with dissections of the same organs post mortem. In most of the hearts there recorded the main axis of the left ventricle lies about 45° from the longitudinal axis of the body. The axis of the right ventricle deviates about 20° from the body's axis on the opposite side. Inspection of a large number of orthodiagrams with the same problem in mind gave the impression that the position of the left ventricular axis was usually at 45° but might often vary from 30° to 55°; the right from 35° to 5°. After inspection of his orthodiagram we accepted the values of 45° and 20° for the deviations of the axes of the cardiac chambers of subject Sta.

The maximum possible impacts of the blood in the heart would occur if all the units to be ejected formed a column whose end presented at the

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Fig. 1. A. Schema of the aorta of subject Sta. Cardiac blood units grouped according to the "concentric" hypothesis.

B. Schema of the pulmonary artery of Sta. Cardiac units grouped according to the "column" hypothesis.

C. Solid line—Machella's (1936) blood velocity curve drawn to linear coördinates. Broken line—longitudinal velocity of blood Unit A 1 during a single systole. Derivation of instants of time in text.

D. Longitudinal acceleration and force curves for Unit A 1.

E. Derivation of longitudinal velocity of blood Unit LH 3 during its course from heart into aorta during a single systole.

F. Representative force curves from blood units in the aorta aligned according to the pulse wave velocity.

G. Similar force curves from the pulmonary artery.

H. Force curves from blood units in the left heart according to the "concentric" hypothesis.

I. The same as H, according to the "column" hypothesis.

J. to M. Curves which are the resultant of forces generated by the blood in different anatomical positions.

N. Resultant of all forces, theoretical ballistocardiogram according to the "concentric" theory.

O. Resultant of all forces, theoretical ballistocardiogram according to the "column" view.

outlet valves in the manner shown in figure 1, B. In such a case all the units to be ejected, and another to fill the space about the valves, would start together at the beginning of systole. The resulting impacts have been calculated and the curves have been given in figure 1, I.

The minimum possible impacts caused by blood in the heart would occur if the units to be ejected were arranged in more or less concentric layers, as illustrated in figure 1, A. When the heart starts to contract Units 5 and 6 move off in the current, but Units 1, 2 and 3 are out of it for a while. Much of the initial motion of Units 1, 2 and 3 is along opposite radiae, so that little impact results until each unit, leaving position LH 4 in space, begins to enter the current, when it is strongly accelerated. In this arrangement the units to be ejected do not start their longitudinal motion simultaneously but seriatim, and therefore they do not give their impacts at the same time, as in the "column" theory, but in series. In this calculation, the motion of the units before they entered the current was neglected. The results are shown in figure 1, H.

The "concentric" conception seems to us much closer to the truth than the column theory, but nevertheless it may not be quite correct. The centers of mass of the units in the heart cannot be located in the same place. But any tendency to headward movement of the units before they enter the current would be opposed by the downward movement of the base of the heart during systole, so we have no hesitation in neglecting their impacts until the units enter the current.

Summation of impact curves. The families of impact curves, derived from the motion of blood in the aorta, pulmonary artery and heart can now be combined by adding their ordinates at similar intervals of time. Such resultant curves are due to motion of the blood, and motion of the body will be equal and opposite. So these curves must be multiplied by -1 to give the theoretical ballistocardiogram.

Our method of giving numerical value to the coördinates of such a curve for subject Sta. has been given in detail (Starr et al., 1939) and need not be repeated. The present calculation differs in only one particular, in this instance a mass factor of 1 represents the mass of 1 unit of blood (i.e., 10 grams) while in the previous calculation the mass unit represented the output of both sides of the heart, 120 grams. Hence the sums of the ordinate values of our families of curves must be divided by 12 to make them comparable to curve A 4 of figure 5 of our previous paper (Starr et al., 1939). This has been done and the divisions of ordinate in figure 1 correspond to those used in 1939.

Relation of the curve derived theoretically to that recorded. The shape of the theoretical ballistocardiogram (fig. 1, N and O) resembles the normal records. Due to difficulty in placing the zero line the areas of wave I plus wave J are a better index of size than either alone. When the cardiac

impacts are calculated as maximal, using the column assumption, the area of wave I + wave J in the total theoretical impact curve for Sta., is 34 per cent larger than the average found in his ballistocardiogram; if the cardiac impacts are calculated as minimal, using the concentric assumption, the resultant curve is 24 per cent too small. Obviously by combining the two views a theory could be found which would fit our data exactly. But we are inclined to accept the concentric view as approximately correct and other methods of improving the fit have occurred to us. Increasing the mass of blood assigned to the aorta would have this effect, and there is evidence that Bazett's data underestimate the aortic size (Cournand and Ranges, 1941). Also the impacts from peripheral blood have been neglected hitherto. While blood whose longitudinal velocity is retarded by diversion from the descending aorta to the segmental vessels has been calculated to give a small impact, driving the body feetward at the time of the "J" wave, this would surely be overbalanced by the inclusion of impacts from blood in the head, arms, and legs whose sum would provide a headward impact at this time. Assuming that the velocity curve retained its shape in the periphery, we need the impacts of 45 cc. of peripheral blood to secure agreement when the concentric theory is employed. This does not seem unreasonable but we doubt if further speculation is profitable; for the agreement between theory and fact shown by the curves of figure 1, N and O, is as good as we have a right to expect considering the assumptions involved.

We are now in a position to assign parts of the total impacts to the movement of the blood in different anatomical positions and the results are given in figure 1, J to M. By far the largest part of the impacts we record comes from blood while in the aorta, a conclusion similar to that reached independently by Hamilton (1941). The contribution from blood in the pulmonary artery is much smaller. The footward I wave is largely cardiac in origin, the headward J wave is from aortic blood. We have other support for the cardiac origin of the I wave. In figure 3, D, is shown the record obtained from a patient whose heart, because of the operative removal of the left lung, was found by x-ray to be extremely displaced, the apex beat being 4 cm. below the axillary fold. When the diaphragm is up and the heart almost transverse in position, the I wave is hardly detectable. In this situation the cardiac recoil, delivered transversely, is not recorded. The feetward thrust, due to the longitudinal acceleration of the blood turning from the heart into the aorta, occurs throughout systole and so is largely buried in the headward "J" wave.

Changes in our theoretical conceptions. We formerly believed (Starr et al., 1939) that the normal pendulum movements of the cardiac apex might make considerable difference to our records and so be a major source of error in the estimation of cardiac output. We have changed our view

for two reasons. Not only does the blood in the heart itself make a comparatively small contribution to the impacts but the axes of the two ventricles converge towards the midline, so that small pendulum movements while throwing one axis further from the longitudinal would bring the other more into line. Only in extreme pathological displacement of the heart can any abnormality of form be seen in our records which we can attribute to shift in its position.

We previously believed that abnormal dilatations of the aorta would make a major error in our estimation of cardiac output and we were surprised to find that the presence of an aneurism made no recognizable difference in the ballistic records (fig. 3, E). We now see several possible reasons. Clots filling the abnormal lumen would prevent any reduction of velocity. Also short changes of aortic diameter would have little effect, the momentum lost when diameter is increased being regained when it is decreased and vice versa. However, if the aorta entering an aneurism had a different direction from that leaving it an abnormality might be introduced into the record.

Measurements made from x-ray pictures of aortae visualized in the living by intravenous injections of contrast media by Cournand and Ranges (1941) indicate that our estimates of aortic size from Bazett's compilations (1935) of Suter's (1897) autopsy data yield a figure which is somewhat too small. Data obtained from living subjects being infinitely preferable we will change our figures for aortic section area as soon as the newer data are available. The change will have the effect of raising the estimation of cardiac output somewhat and so bring the average of our results obtained on normal persons closer to corresponding data obtained by other methods. While the absolute value of our results will be altered the significance of changes and deviations from the normal will be unchanged.

The effect on the ballistocardiogram of changes in form of the blood velocity curve has been recalculated by assuming a curve in which maximum velocity is attained late in systole, the mirror image of the velocity curve in figure 1, C, and then using the methods described herein. The results look so much like the curves published before (Starr et al., 1939, fig. 5) that they will not be repeated here.

To study the effect of changes in pulse wave velocity we recalculated the impacts of Sta., substituting the pulse wave velocities obtained by Bazett (1941) on an old man: heart subclavian, 5.6; and subclavian to femoral, 11.7 meters per sec. The resulting impact curve could hardly be distinguished from the one in which the average values of his age group, 3.3 and 7.6 meters per sec., were used. So we believe that physiological changes of pulse wave velocity will not make a detectable difference in the ballistic records.

Our first theoretical approximation (Starr et al., 1939) explained the

impacts as the resultant of three curves. The second of these curves was attributed to the arrest of the blood moving headward by the aorta arch and the backward curve of the pulmonary artery. This explanation belongs much better to the second part of the first curve. We now realize that the second and third curves have a common origin; they chiefly represent the impacts due to the movement of the large mass of blood in the descending aorta.

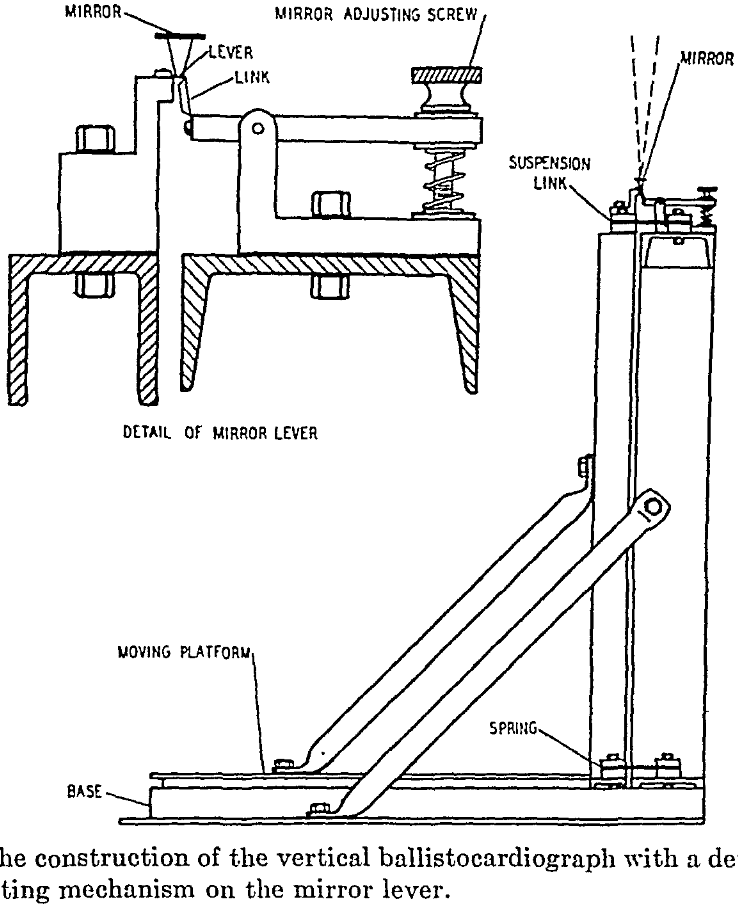

Fig. 2. The construction of the vertical ballistocardiograph with a detail drawing of the adjusting mechanism on the mirror lever.

In spite of these numerous improvements and clarifications in our views, this study supports all the main features of our previous conceptions and we have nothing further to add either to our method of calculating cardiac output from the records or to our interpretation of abnormal forms.

Apparatus and records. As indicated in figure 2 the vertical ballistocardiograph consists of a light weight Duraluminum platform attached to a vertical steel frame which is flexibly connected to a heavy steel base frame, so that the platform can only move in a vertical direction. This vertical movement is restrained by two stiff flat steel springs whose tension can be adjusted by altering the length in use. Relative movement be-

tween the platform and the base frame drives a mirror mounted on a short lever, thus deflecting a light beam. This deflection is recorded on a standard photo-kymograph.

Difficulty was encountered obtaining a record free of serious distortion from vibrations in the building largely due to the elevators. These vertical vibrations ruined the record when the vertical instrument was placed directly on the floor and simple measures such as mounting it on rubber were ineffective. It was necessary to place 500 lbs. of steel plates under the base frame and support the whole on elastic material of diverse properties. One edge is supported by a ridge of rubber-like material 3 cm. wide by 5 cm. thick, the opposite edge by a row of 12 tennis balls compressed hard, the center by about 60 tennis balls compressed lightly. The result is not perfect, for vibrations of a frequency averaging about 13 per second disturb the record repeatedly, for periods of a few seconds each, whenever the elevators are in use.

Calibration. The springs have been adjusted so that 280 grams placed on the platform displaces the light spot image 1 cm. which makes the calibrations of both horizontal and vertical ballistocardiograms the same.

Period of vibration. When weighted with iron and struck a single blow the frequency is as follows: at 100 lbs., 22 vibrations per sec., at 150 lbs., 11.5 per sec., at 200 lbs., 10.5 per sec. Thus the frequency of the vertical instrument is almost 80 per cent faster than the horizontal at 100 lbs., but only about 10 per cent faster at 200 lbs.

Vibrations in the vertical human body. Thirteen subjects, standing on the platform, were struck a series of taps on the head or shoulder. Analysis of those records which chanced to fall between cardiac complexes showed that the vibrations set up had an average frequency of 5.57 per sec. But the range was from 5 to 6.7 per second in individual subjects and this average is not significantly different from 5.72 per sec., the corresponding average found in horizontal subjects.

After such taps had deflected the light spot, on its return it overshot the base line by an amount which averaged 65 per cent of the height of the previous deflection. This is significantly different from the corresponding average of 40 per cent found in horizontal subjects. The body provides more damping in the horizontal than in the vertical position.

Taking records. As subjects depress the platform a different amount depending on their weights, the mirror adjusting screw must be used to direct the light spot to the camera after each change of subject. Then the operator watches the flickering image, chooses a time when distortion is at a minimum and takes the photograph.

Measurement and calculation from the records. The area method (Starr et al., 1939) has been used to calculate cardiac output. Typical large and small complexes of the respiratory cycle were selected. The line on the

record has considerable width and in making all measurements we used the top edge. A base line was drawn at the position this edge would occupy if the heart were not beating. With this base line waves I and J formed areas which were almost triangular, the first below, the second above the base line. With a ruler and sharp pencil true triangles were superimposed, the aim being to shave off from the sides as much area as was added at the apex. The bases of these constructed triangles were measured in fractions of a second, their altitudes in millimeters. The values obtained in the two selected complexes were averaged and cardiac output was estimated as described before (Starr et al., 1939).

Statistical methods. The methods of Fisher (1938) were employed. The word significant is always used in the statistical sense, indicating a probability of 0.05 or less that the difference is due to chance.

RESULTS. *Comparison of ballistocardiograms of vertical and horizontal subjects.* Typical "V" records are shown in figure 3 and they show systolic complexes of a smaller amplitude than the corresponding "H" records. This is in accord with the diminished stroke volume found in the erect posture by all other cardiac output methods. The time relations to the electrocardiogram are the same in "V" and "H" records (fig. 3, C).

In normal subjects the systolic complexes have a *similar form* in both "H" and "V" records. To investigate the finer details, the records of 20 subjects tested in both positions were chosen at random; the average durations of the "H" and "V" downward deflections, I waves, were 0.058 and 0.056 sec. respectively; the upward deflections, J waves, 0.091 and 0.096 sec., differences well within the error of placing the base line.

Nevertheless *major differences* usually permit the recognition of "V" records at a glance. Due to shifting of the subject's weight from foot to foot the base line may wander. Deflections in diastole are often higher in relation to the systolic complexes, so that the latter do not stand out as clearly as in "H" records. When the elevators start or stop rapid vibrations at a frequency averaging 13.5 and ranging from 10 to 15 per second, often confuse the "V" records for several seconds. Interfering vibrations of this frequency are seldom, if ever, seen in "H" records. Muscle tremors, brought out by the use of the muscles in standing, may confuse or destroy the usefulness of the "V" record, while the "H" record is unaffected (fig. 3, I).

EXPERIMENTS. *Subjects.* The normal subjects used were either medical students, young doctors, or hospital and laboratory technicians. There were 36 men and 22 women all over 18 and under 35 years of age except the first author. No test was made within 2 hours of a meal.

Technique. The subject lay relaxed on the horizontal table for 15 minutes or longer. At the end of this period blood pressure and ballistocardiogram were taken. He then arose and took several steps to the vertical

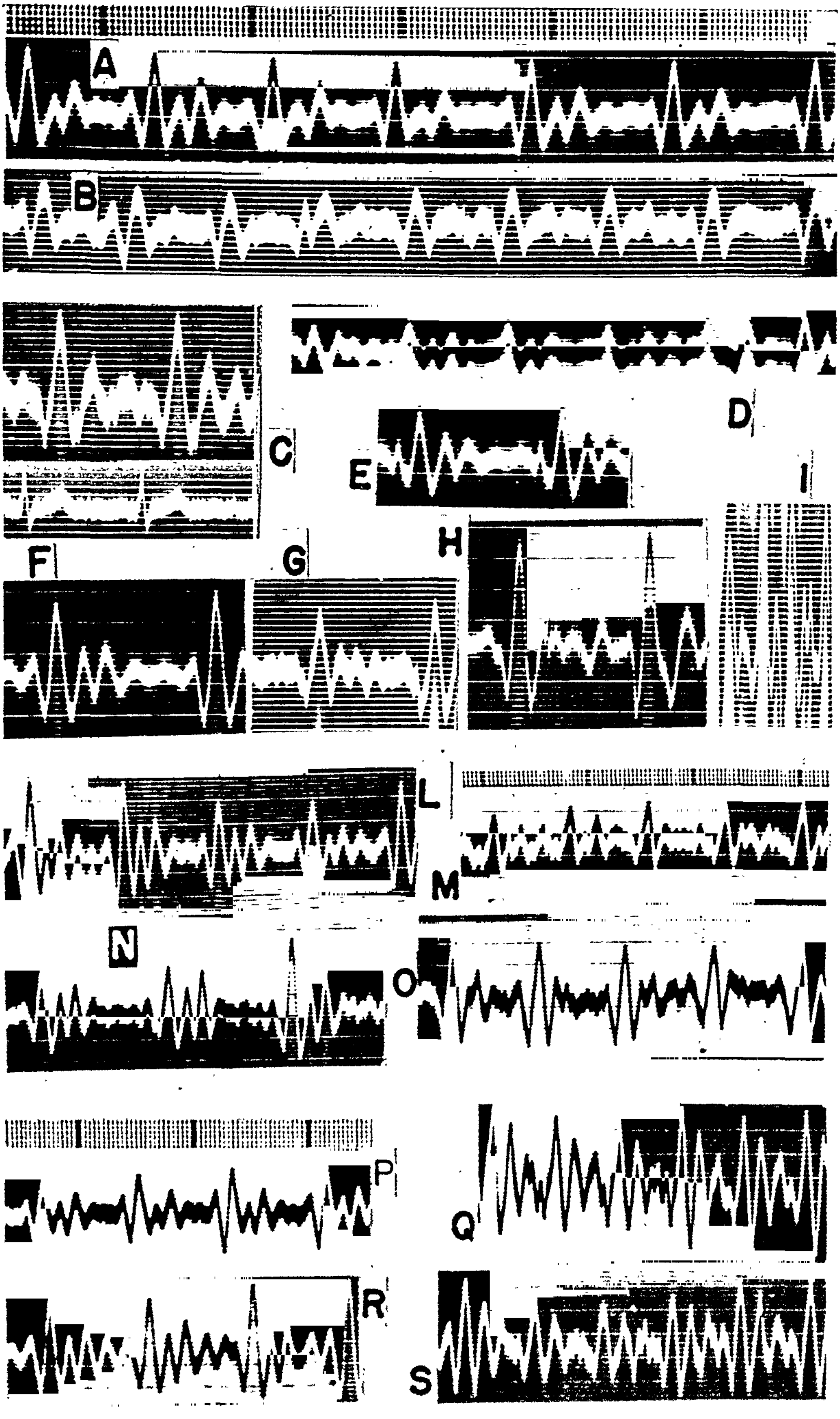

Fig. 3

ballistocardiograph which stood at the foot of the horizontal instrument, and stepped up 9 inches to its platform, on which he stood relaxed with his feet either together or apart, records being taken 1 and 2½ minutes after taking his stance in most experiments, with additional observations at 5, 10 and 15 minutes in a few. Prolonged standing was not studied.

Fig. 3. Ballistocardiograms of subjects in the vertical and horizontal positions.

A. to I. Reproductions approx. actual size. Time record over A applies to all. Largest interval 1 sec.

A. Subject M. L., age 34, 5 ft. 3 in., 124 lbs. A normal woman after 15 min. rest horizontal.

B. Same after standing 2½ min.

C. Vertical ballisto. of H. E., age 24, 5 ft. 11 in., 158 lbs. Male. Lead 1 of the electrocardiogram recorded simultaneously on the same film.

D. Horizontal ballisto. of E. W., age 28, 5 ft. 4 in., 114 lbs. Female. Six months after left pneumonectomy. X-ray shows the heart in contact with the left chest wall; the apex impulse is in the axilla. Note absence of initial downward deflection (I wave) in smaller complexes.

E. Horizontal ballisto. of R. M., age 58, 5 ft. 8 in., 133. Male. Large aneurysm of ascending and transverse aorta. Note normal record in spite of the huge dilatation of the aorta.

F. S. D., 24, 5 ft. 6 in., 132 lbs. A normal medical student after resting 15 min. horizontal. B.P. 112/82.

G. Same after standing the next 2½ min., B.P. 114/90.

H. Same lying 10 minutes after receiving 0.5 cc. adrenalin s.c. B.P. 140/68.

I. Same standing next 2½ min. Note that the adrenalin tremor destroys the vertical record while the horizontal is affected little if at all.

L. to O. Effect of Paredrine. Records reduced to two-thirds actual size. Time record over M applies to all this group. Subject E. W., normal medical student, age 23, 6 ft. 1 in., 155 lbs.

L. After 15 min. rest horizontal. Cardiac output per minute 26 cc. per min. per lb. body weight, + 13 per cent of average normal. B.P. 120/80.

M. Same subject after standing 2½ min. C.O. = 24 cc. B.P. 122/88.

N. Same subject lying ½ hr. after receiving 20 mgm. N-methyl paredrine hydrochloride subcutaneously. C.O. = 21. B.P. 168/110.

O. Same subject after standing next 2½ min. C.O. = 29 cc. B.P. 146/96. Note that after paredrine the response of the cardiac output to arising was reversed.

P. to S. Effect of an abdominal binder. Records reduced to three-quarters actual size. Time record over P applies to all this group. Patient W. T., age 50, 5 ft. 6 in., 128 lbs. Complains of frequent attacks of faintness when standing.

P. After 15 min. rest. Cardiac output = 28 cc. per min. per lb.; + 22 per cent of average normal. B.P. 90/60.

Q. After standing next 2½ min., feeling dizzy and faint. C.O. = 40 cc. B.P. 88/70.

R. After 15 min. rest with abdominal binder in place. C.O. = 34 cc. B.P. 95/65.

S. After standing next 2½ min. with binder. No symptoms. C.O. = 33 cc. B.P. 95/75. A vibration in the building affects the center of this record. Note that dizziness occurred in the presence of an abnormally large cardiac output per minute and was absent when this was smaller. Also the presence of the binder changed the response of the cardiac output to arising.

The plateau after arising. Records started as soon as the subject reached the platform showed diminishing impacts for a brief period which we interpreted as the effects of the exertion passing off. After one minute of standing a plateau was attained, for the averages show no significant difference for the next 10 minutes in the 3 subjects tested (table 1). However, after 15 minutes' standing the averages showed a very slight but significant increase in cardiac output in these three subjects that may well be correlated with the increased restlessness often seen at this time. Duplicate estimations made in every experiment at 1 and $2\frac{1}{2}$ minutes, or $2\frac{1}{2}$ and 5 minutes, after assuming the erect posture confirmed the impression that

TABLE 1

Changes in cardiac output per minute during 15 minutes' quiet standing

DATE	SUBJECT	SEX	AGE	CHANGE FROM VALUE FOUND AFTER $2\frac{1}{2}$ MINUTES' STANDING		
				5 minutes	10 minutes	15 minutes
				per cent	*per cent*	*per cent*
4-19-40	Sta.	M	45		−5	+5
4-29-40				+9	+4	+16
6- 3-40				0	+5	+5
1-21-41				−10	+4	+4
4-24-40	C. H.	F	26	−7	+4	−11
5- 1-40				−18	−11	+11
5-10-40				+15	+30	+15
5-31-40				−5	+10	+20
12-24-40	M. T.	F	25	−5	−5	−10
1-20-41				+5	−10	+5
Averages				−2	+2.6	+6
Significant for P = 0.05				No	No	Yes

the circulation was steady during this period. As records taken at $2\frac{1}{2}$ minutes are common to all experiments, only these results will be reported.

Changes in the circulation on arising. Fifty-six subjects performed the test for the first time and the results are recorded in table 2. The changes of pulse rate and blood pressure which occurred are so familiar that only the averages have been given in table 2. The average cardiac output per beat diminished significantly; the average cardiac output per minute was unchanged.

The average of these results contrasts with those obtained on the first author and his two technicians in tests made at monthly intervals (table 3). The latter two subjects showed the same cardiac output per minute stand-

TABLE 2

Relation of cardiac output per minute after resting 15 minutes recumbent and after standing the next $2\frac{1}{2}$ minutes at rest

First test on each subject. Cardiac output given in per cent deviation from average normal which equals 51 cc. per min. per kilo body weight, or 23 cc. per min. per lb., when Bazett's data (1935) on aortic size are used. Normal limits ±22 per cent.

SUBJECT	SEX	CARDIAC OUTPUT PER MINUTE: When horizontal	CARDIAC OUTPUT PER MINUTE: Change on standing	SUBJECT	SEX	CARDIAC OUTPUT PER MINUTE: When horizontal	CARDIAC OUTPUT PER MINUTE: Change on standing	SUBJECT	SEX	CARDIAC OUTPUT PER MINUTE: When horizontal	CARDIAC OUTPUT PER MINUTE: Change on standing
		per cent deviation from normal average	*per cent*			*per cent deviation from normal average*	*per cent*			*per cent deviation from normal average*	*per cent*
Ho.	M	+35	−10	Sy.	F	+4	−13	Lo.	M	−9	−9
Cal.	F	+22	−5	Ro.	M	+4	−12	Co.	M	−9	+14
Cr.	M	+22	+11	Am.	M	+4	−4	Cap.	M	−9	+14
We.	M	+17	−4	La.	M	+4	0	Al.	M	−9	+19
Ha.	M	+17	0	Do.	M	+4	+8	Koe.	M	−9	+38
Ho.	F	+17	+4	Jo.	F	0	−17	Ros.	M	−13	−20
Br.	F	+17	+4	He.	M	0	−17	Ki.	M	−13	0
Ja.	M	+17	+11	To.	F	0	+4	Ze.	F	−17	−4
Ji.	F	+13	−15	Erb.	M	0	+4	Calk.	F	−17	+4
Ev.	F	+13	−15	Po.	M	0	+13	Ca.	M	−17	+16
Con.	M	+13	−12	Fr.	M	0	+22	Ul.	M	−17	+5
Wi.	M	+13	−8	Bra.	M	0	+22	Ir.	F	−17	+10
Er.	M	+13	+4	De.	F	−4	−25	Ha.	F	−22	0
Fra.	F	+13	0	Vi.	M	−4	−18	Ka.	M	−22	+6
El.	F	+9	−32	Ko.	F	−4	+15	Na.	M	−22	+11
Ir.	M	+9	−16	Mc.	F	−4	+27	Ba.	F	−22	+17
My.	M	+9	−18	Be.	F	−4	+14	Ri.	M	−22	+17
Mu.	F	+9	−12					La.	M	−30	+6
Pa.	F	+9	+8								
Iro.	M	+9	+16								
Pas	M	+9	0								

Average changes on assuming the erect position. Means and standard deviations about the means

	MEANS	STANDARD DEVIATION	SIGNIFICANCE		MEANS	STANDARD DEVIATION	SIGNIFICANCE
					mm. Hg	*mm.* Hg	
Cardiac output per minute....	+1%	14%	No	Systolic B.P.......	+1.1	7	No
Stroke volume..	−17.6%	9.6%	Yes	Diastolic B.P......	+6.4	8	Yes
Pulse rate.......	+16.6 per min.	8.3 per min.	Yes				

TABLE 3

Relation of cardiac output per minute after resting 15 minutes recumbent and after standing the next 2½ minutes

Repeated tests in single subjects none of whom were originally accustomed to the test, except Sta.

SUBJECT	DATE	CARDIAC OUTPUT PER MINUTE: When horizontal	CARDIAC OUTPUT PER MINUTE: Change on standing	SUBJECT	DATE	CARDIAC OUTPUT PER MINUTE: When horizontal	CARDIAC OUTPUT PER MINUTE: Change on standing	SUBJECT	DATE	CARDIAC OUTPUT PER MINUTE: When horizontal	CARDIAC OUTPUT PER MINUTE: Change on standing
		per cent deviation from normal average	*per cent*			*per cent deviation from normal average*	*per cent*			*per cent deviation from normal average*	*per cent*
Sta.	4–19	+17	−19	Be.	2–4	−4	+14	Calk.	3–14	−17	+4
	4–23	+13	−10		2–18	−4	0		3–28	−9	−12
	4–29	+22	−18								
	6–3	+9	−12	Ha.	2–7	−22	0	Pa.	3–21	+9	+8
	7–15	+4	−21		2–17	−25	+8		3–25	0	−6
	11–18	+17	−18								
	12–24	+9	−12	Ze.	2–10	−17	−4	Mu.	3–21	+9	−12
	1–21	+4	−13		2–18	−25	−6		3–25	+30	−24
	2–27	+22	−11								
	3–21	+30	−13	Jo.	2–11	0	−17	Li.	3–22	−9	+31
					3–17	−39	0		3–24	0	−9
Ho.	4–24	+17	+4								
	5–1	+29	−7	Ba.	2–14	−22	+17	De.	3–24	−4	−25
	5–10	+24	−17		2–27	−13	−5		4–1	−9	−14
	5–31	+25	−20								
	6–4	+25	+8	Sy.	2–28	+4	−13	Ir.	3–27	−17	+10
					3–11	−17	+5		4–1	−13	0
To.	11–19	0	+4								
	12–24	+13	−19	Cal.	3–1	+22	−5	Ji.	4–22	+13	−15
	1–20	+22	−29		3–15	+26	+14		4–28	+13	+4
	2–27	+9	−8								
	3–21	0	−14	Ri.	3–10	−22	+17	El.	4–22	+9	−32
					3–20	−13	+10		4–28	+9	−12
				Ko.	3–11	−4	+15				
					3–27	−4	+5				

Average change on standing, first test. . . +2% Difference not significant
Average change on standing, second test. −5%

ing as lying the first time they underwent the experiment but on most subsequent tests they exhibited a smaller circulation when erect. The first author, a veteran subject, always showed a smaller circulation when

erect. Therefore we suspected that slight emotion incident to the performance of the test for the first time might play a part in the results.

Accordingly 20 subjects who had had no previous experience on the apparatus were given 2 tests about a week apart and the results are recorded in table 3. The average change in the cardiac output per minute, on assuming the erect position, was +2 per cent at the first test and −5 per cent on the second, a difference in the direction expected but not of statistical significance.

Agents altering the response to arising. In the course of class demonstrations of the action of drugs in normal medical students, records were obtained at the end of alternate periods of lying 10 or 15 minutes, and standing $2\frac{1}{2}$ minutes, both before and during drug action. Sixty such experiments were made. The striking feature of the results was the demonstration that in certain types of drug action the circulation in the horizontal position might be affected in one direction, while in the vertical position this effect was reversed. The most striking examples occurred in experiments after n-methyl paredrine hydrochloride[1] and one series of such records is given in figure 3. At a time when the drug had caused some slowing of the pulse rate and consequent diminution of cardiac output per minute when the subject was horizontal, as found by Altschule and Iglaver (1940), the circulation was strongly stimulated when he stood erect. This effect was obtained on all 8 subjects given the drug, and the accelerated circulation in the erect posture showed no tendency to diminish for 10 minutes after arising; longer periods were not tested.

Most of the drugs tested affected the circulation similarly in the two positions, and while a diverse effect was seen occasionally it was never of the degree or with the consistency seen after paredrine. Therapeutic doses of adrenalin regularly increased the circulation in both positions, but in one subject as its action was passing off, the increase was found greater in the horizontal position. Eight milligrams benzedrine caused a similar effect on one occasion. Histamine gave inconsistent results; on one occasion 0.35 mgm. increased the circulation when the subject lay without affecting it as he stood, while on another 0.4 mgm. increased it in both positions. Nitroglycerine gr. $\frac{1}{50}$ under the tongue always increased the circulation in both positions. Therapeutic doses of strychnine, metrazol, coramine, atropine, pitressin, and prostigmin, and distilled water, all given subcutaneously, had no noteworthy effect on the amount of the circulation in either position.

If the abdominal wall is relaxed the application of a binder often increases the horizontal circulation but diminishes the vertical, as is shown

[1] We are indebted to the Smith, Kline, and French Laboratories for the drug employed.

in figure 3. But in most normal subjects a binder causes little change in either position.

Symptoms of faintness and the general circulation. On six occasions normal subjects complained of transient symptoms of faintness, lightheadedness, or dizziness while standing on the vertical ballistocardiograph. In three instances the symptoms occurred spontaneously, in the remainder they occurred during drug action, especially after nitroglycerine placed under the tongue. In no case was the cardiac output smaller during the symptoms than in corresponding periods when the patient was symptom free. On the contrary, the symptoms were often experienced during a period in which the cardiac output was definitely greater than that existing when they were absent (fig. 3, Q). A slight diminution of blood pressure was usually, but not always observed during these symptoms, but in no case was the remaining pressure insufficient to raise blood to the top of the head. Schneider and Crampton (1934) and also Schellung and Heinemeier (1933) reported similar findings and these results were expected by Starr and Collins (1931b).

Discussion. The ballistocardiograph is not to be regarded as an instrument of high accuracy for the estimation of cardiac output. Nevertheless the general agreement of the results with those obtained by other methods has been confirmed (Cournand and Ranges, 1941). In the light of 5 years' experience the internal evidence continues very good; i.e., duplicates agree well, and agents known to increase cardiac output, such as exercise and certain drugs, increase the size of the impacts invariably. The method gives most reasonable results; that it is an easy qualitative measure of changes of cardiac output cannot, in our opinion, be disputed; and this is sufficient justification for its use. But its possibilities are far greater than this because, by means of Newton's "Laws of Motion," the record is mathematically related to fundamental cardiac functions, the amount of blood ejected and the manner of its ejection. Assumptions are necessary to estimate this relationship; it is our hope that further work will increase our knowledge of them. The absolute quantitative accuracy of this, and the other cardiac output methods, is unknown.

The number of assumptions necessary varies with the nature of the experiment. Thus to compare the cardiac output of different subjects the size of their great vessels must be estimated; to compare the cardiac output of the same subject under different conditions, as in this investigation, this is not necessary.

Cardiac outputs estimated by ballistocardiograms can be directly compared only if the manner of ejecting blood from the heart, i.e., the curve of blood velocity during ejection, remains normal. We have evidence (Starr et al., 1938) that changes in the form of this curve would alter the form of the ballistic record so that the presence of this abnormality could

be recognized. No such changes occurred during these experiments, although distortions of form, similar to those calculated from abnormal blood velocity curves (Starr and Schroeder, 1940) are common in persons with damaged hearts.

Our vertical ballistocardiograph is distinctly inferior to our horizontal instrument. The former is much more difficult to insulate from vibrations due to the 3 elevators in our steel and concrete building; more primitive housing might obviate this difficulty in other places. Its record is much more likely to be completely ruined by muscular tremors. The advantage of the horizontal position was appreciated by Gordon (1877) who seems to have been the originator of this field (Lamport, 1941). The investigations of Abramson (1933) and Heald and Tucker (1922) must have been handicapped by the fact that they used vertical instruments.

Some types of investigation cannot be performed. Patients subject to fainting placed on the platform have developed uncontrollable muscular movements which ruined the record long before any symptoms set in. Patients who are weak from any cause, or who have been long in bed, usually can not stand still enough to give usable "V" records, although their records when horizontal may be entirely satisfactory. Nevertheless satisfactory "V" records can be obtained on almost all normal persons and on a majority of ambulatory patients.

The average relation between the cardiac output lying and after standing 15 minutes or longer has been disputed, some authors finding a diminution, others no change (McDowell, 1938). The same discrepancy appears in our results for, while our averages show no change, the circulation of certain subjects as Sta. (table 3) regularly diminishes in the erect posture. Our attempt to explain the difference as the result of becoming accustomed to the test yielded suggestive evidence, but failed to prove the point, so we sought for other explanations. Another factor became obvious as soon as the results were arranged in order of magnitude as in table 2. Subject Sta. is one of many whose cardiac output per minute, when lying, was above the average normal, and who showed a conspicuous diminution of the circulation on arising. With few exceptions it is those whose cardiac output per minute, when recumbent, is below the normal average who show no change, or an increase, when they arise. There is significant correlation between the original level of the cardiac output when lying and the change on arising.

The search for similar correlations in the literature was handicapped by the failure of a number of authors to report the weight of their subjects. In our data the relation is significant only when weight is taken into account. Nevertheless, in the data of Fisher (1932) and Donal, Gamble and Shaw (1934) both the absolute value of the cardiac output per minute, and the cardiac output per minute related to body weight, showed

significant correlation with the change on arising, so we have not disregarded the data when weight was not given.

In table 4 the results of 4 authors using the same method are arranged in order of magnitude. The strong positive correlation between the level

TABLE 4

Data on the change of cardiac output in lying and standing subjects, obtained from McMichael (1937); Schneider and Crampton (1934); Grollman (1932); and Goldbloom, Krause and Lieberson (1940); and arranged in order of magnitude of cardiac output when lying

AUTHORS	SUBJECT'S CARDIAC OUTPUT LYING	CHANGE ON STANDING	AUTHORS	SUBJECT'S CARDIAC OUTPUT LYING	CHANGE ON STANDING	AUTHORS	SUBJECT'S CARDIAC OUTPUT LYING	CHANGE ON STANDING
	liters per minute	*per cent*		*liters per minute*	*per cent*		*liters per minute*	*per cent*
S & C	6.5	−22	G.	4.3	−19	G. K. L.	3.8	+9
McM.	5.8	−33	G.	4.3	−16	G. K. L.	3.8	+3
McM.	5.3	−21	G.	4.3	−9	McM.	3.7	+30
S & C	5.2	−29	G.	4.3	−2	G. K. L.	3.6	+5
S & C	5.0	−26	S & C	4.2	−10	G.	3.5	0
McM.	5.0	−14	G.	4.1	−7	McM.	3.5	0
McM.	4.9	−25	G.	4.1	0	G.	3.2	0
McM.	4.8	+2	G. K. L.	4.1	+4	G.	3.2	+3
McM.	4.7	−34	G.	4.0	−5	McM.	3.0	0
S & C	4.4	−18	G. K. L.	4.0	0	McM.	2.6	+4
McM.	4.3	−23	McM.	3.9	−3	G.	2.4	+4

TABLE 5

Relationship between the magnitude of the cardiac output per minute with the subject recumbent and the change on arising in per cent of the recumbent value

AUTHORS	NUMBER OF TESTS	CARDIAC OUTPUT ABSOLUTE VALUE, OR REFERRED TO BODY WEIGHT	LEVEL BELOW WHICH CORRELATION IS NOT SIGNIFICANT FOR P = 0.05	CORRELATION COEFFICIENT
Starr and Rawson (this paper)	56	body wt.	0.26	0.30
Donal, Gamble and Shaw	23	body wt.	0.40	0.42
Fisher	47	body wt.	0.28	0.57
4 Authors of table 4	33	abs.	0.33	0.69
Schellong and Heinemeier	28	body wt.	0.36	0.41
Nylin	11	body wt.	0.55	−0.18
Bock	17	abs.	0.45	0.46

of cardiac output of the recumbent subjects and the change on standing is obvious at a glance.

The statistical results are given in table 5. With the exception of a short series by Nylin (1934), all the series long enough to make statistical

analysis worth while, and obtained by cardiac output methods still in use, demonstrate the correlation mentioned above. But in the absence of a statistical analysis the fact has not been realized.

This conception resolves some of the discrepancies in the literature for the results of Schneider and Crampton (1934), believed to be at variance with those of Grollman (1932) because the averages were different, are now seen to fit well with the other data when its larger relations are discovered. Most of Grollman's (1932) subjects were in the basal condition; none of Schneider and Crampton's (1934) were basal, so the resting cardiac output was larger in the latter investigation.

Thus some of the diversity of recorded result can be explained by the experimental conditions employed or by chance in the selection of subjects, but there are surely other factors. Most of these studies differ from ours in that, since estimation of cardiac output by the methods previously available required considerable time, the interest necessarily centered on the effects of standing for longer periods than we usually employed. Prolonged standing provides time for the accumulation of blood and lymph in the dependent parts of the body and so brings into play a slow physiological mechanism not important in the majority of our experiments (Asmussen et al., 1939).

Other errors must be present, for cardiac output methods are crude. The contrasting experiences of Stewart and Cohn (1932) and Harrison (1939); and of Grollman (1932) and Gladstone (1935); and the evidence of Sweeney and Mayerson (1937), indicate that small differences in the duration of rebreathing can make no little difference in the results of those methods which aim to secure gaseous equilibrium in the lungs before the blood returns a second time. So experimental error and slight differences in experimental technique are surely additional factors in the diversity of the recorded results. Nevertheless, the conception cited above does much to unify the field and the divergent results do not occur more frequently than one could expect from chance accumulation of the inherent errors.

The published data suggest that there is a limit below which the cardiac output per minute of a standing subject cannot go with safety, and that this limit is located near the middle of the normal range found in recumbent subjects, whose circulations are governed by different and less rigid requirements. If the cardiac output per minute of the recumbent subject exceeds his standing requirement, then the circulation usually diminishes on arising. If the recumbent value does not exceed the standing requirement, there is no change or an increase in the circulation when the subject arises.

Our results show that drugs and other physiological agents acting on the circulation do not necessarily produce the same effect when the subject is lying as when he is standing and so may profoundly modify the response

to arising. Data on the action of such agents in the erect posture is almost non-existent and the apparatus herein described seems well suited to obtain more information.

SUMMARY

Theoretical studies to account for the origin of the ballistic waves have been continued. The major part of the systolic complex is due to the movements of the blood in the aorta.

A ballistocardiograph, designed to secure records in standing or sitting subjects, has been constructed.

By this means we have studied the immediate response of the circulation to change of position, concentrating our attention on the plateau which extends from 1 to 10 minutes after arising.

In normal persons the average cardiac output per minute is the same at this time as before they arose.

On assuming the erect posture, the cardiac output per minute of some subjects regularly diminishes, in others it remains the same, in others it increases somewhat. The original state of the circulation is an important factor in this difference, i.e., when the resting cardiac output is in the upper half of the normal range, it diminishes on arising in the majority of tests; if in the lower half of the normal range, it increases on arising in the majority.

The drug N-methyl paredrine hydrochloride, which diminishes the cardiac output per minute in the supine position, increases it in the erect position, so that the response to arising is profoundly altered. No other drug tested gave effects comparable to this.

The application of an abdominal binder, in certain persons, affects the circulation in different directions in the erect and supine positions.

Symptoms of dizziness or lightheadedness on arising were not coincident with a diminished output of the heart.

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DESOXYCORTICOSTERONE AS A PROPHYLACTIC FORE-TREATMENT FOR THE PREVENTION OF CIRCULATORY FAILURE FOLLOWING HEMORRHAGE AND SURGICAL TRAUMA IN THE ADRENALECTOMIZED DOG[1]

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The synthetic adrenal steroid desoxycorticosterone acetate (D.C.A.) will, in small doses, adequately maintain adrenalectomized animals (1, 2, 3). It will also protect the dog lacking adrenals against circulatory collapse induced by *a*, muscle trauma; *b*, intraperitoneal injection of isotonic glucose; *c*, infusion of massive doses of epinephrine (4). According to Perla *et al.*, D.C.A. and saline protects normal rats against histamine (5). However, it does not prevent the circulatory failure which follows intestinal stripping in the adrenalectomized dog (4).

Selye, Dosne, Bassett and Whittaker (6) and Weil and Browne (7) have reported negative results when D.C.A. was employed in attempts to prolong the survival of intact rats and rabbits subjected to intestinal trauma. Selye and Dosne (8) state that corticosterone, which differs rom D.C.A. by the presence of a hydroxyl group at C_{11}, was effective, and advocated its use in the treatment of traumatic shock in man. The writers (4) have found corticosterone to be an efficient agent in preventing the circulatory collapse resulting from intestinal manipulation in adrenalectomized dogs.

D.C.A. was used in this study.[2] The two procedures chosen to produce circulatory collapse were of qualitatively different nature. One involved the physiological responses to a simple loss in circulating blood volume, i.e., massive hemorrhage; the other, surgical trauma incident to a single stage bilateral adrenalectomy. In the latter procedure blood loss is negligible, but trauma to a not inconsiderable amount of nervous tissue in the immediate vicinity of the adrenal glands is often unavoidable.

METHODS. In the hemorrhage experiments trained, unanesthetized dogs

[1] Part of the expenses of this investigation was defrayed by Julian M. Livingston of New Rochelle, N. Y.

[2] We are indebted to the Ciba Pharmaceutical Products, Inc. for generous supplies of the desoxycorticosterone acetate (Percorten) used in these experiments.

were bled at an approximately uniform rate of 10 cc. blood per minute, continued until the arterial pressure had been reduced to 50–40 mm. Hg. The blood was taken by needle-puncture from the femoral artery, using syringes inserted into a three-way stopcock, with the side arm connected to a fluid trap and sphygmomanometer (9). By merely rotating the stopcock, arterial pressures could be taken at will during the course of the hemorrhage.

The single stage bilateral adrenalectomies were performed under nembutal anesthesia. In certain of these operations, and previous to dissection of the glands for removal, the nerves in the region of the adrenals were locally blocked by means of a 4 per cent solution of procaine hydrochloride in sterile water, prepared from Novocaine (Metz) crystals.

The D.C.A. treated animals were given 20 mgm. (four 5 mgm. doses intramuscularly at 24, 18, 12 and 2 hrs.) prior to the experiment.

The techniques used in blood chemical analyses and arterial pressure determinations have been described elsewhere (10).

I. *The effectiveness of D.C.A in increasing the resistance of the adrenalectomized dog to hemorrhage.* The intact, unanesthetized dog, can withstand the loss of 40 to 54 cc. of blood per kgm. body weight before the blood pressure declines permanently to shock levels (11). If the bleeding is stopped at any stage previous to this, rapid blood dilution and a steady rise in pressure follows. The adrenalectomized dog not receiving extract, still eating full rations, and without obvious signs of physical weakness, but with the arterial pressure lowered by some 20 mm. Hg from normal, cannot withstand the loss of 4 to 8 cc. blood per kgm. body weight without showing an abrupt pressure fall to shock level. In this type of experimental animal, and in sharp contrast to the intact dog, the blood pressure will not spontaneously rise from this level, and death invariably follows within a few hours unless extract treatment is given (11).

The animals subjected to hemorrhage consisted of ten vigorous adrenalectomized dogs: four D.C.A. treated, and six controls. Two of the controls, when bled, were receiving adequate but minimal maintenance daily doses of cortical extract. Four animals served as controls of a somewhat different type, since extract therapy had been withdrawn for a period of 24 hours prior to hemorrhage. During this interval negligible changes had occurred in blood chemistry, body weight, vigor and blood pressure. The blood urea nitrogen was in general slightly elevated, however (table 1).

The blood pressure changes during the course of the hemorrhage in a representative D.C.A. treated animal, and in one from which extract therapy had been discontinued for 24 hours, are shown in figure 1. The first withdrawal of blood was followed, in the D.C.A. treated dog, as in the intact one, by a rise in arterial pressure. This rise was sustained until

some 20 to 28 cc. per kgm. body weight had been withdrawn. The pressure then showed a rapid decline, so that the removal of only 5 to 12 cc. per kgm. body weight additional blood sufficed to produce a lowering to shock levels. In this particular dog, the pressure was normal or above throughout 83 per cent of the total hemorrhage, and then fell 70 mm. Hg with the final 17 per cent.

The dogs receiving maintenance doses of cortical extract showed a much shorter period than the D.C.A. treated animal, during which the pressure was elevated above normal. Thus, only about 10 cc. blood per kgm. body weight could be removed before the pressure fell below normal. An additional 6 cc. lowered it to shock levels. The dogs from which extract had been withheld for 24 hours showed no initial rise in pressure (fig. 1).

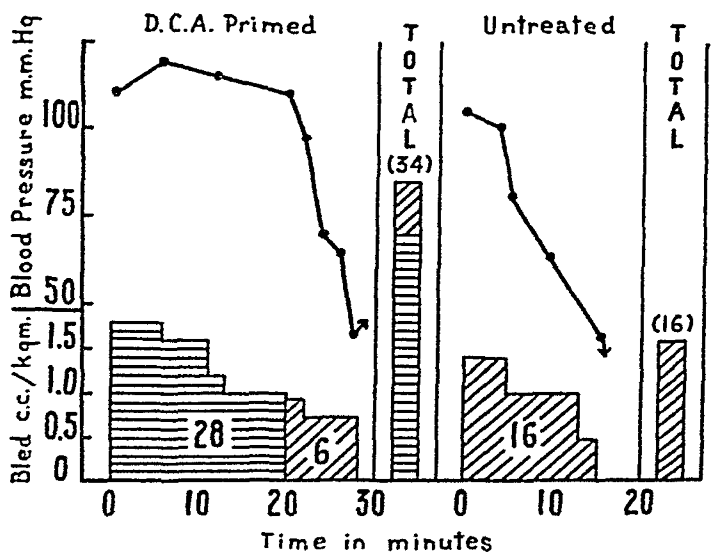

Fig. 1. Effect of hemorrhage in D.C.A. primed adrenalectomised dog

In these animals, therefore, the pressure was falling over the whole course of the hemorrhage. A pressure of 40 mm. Hg was produced by the removal of only half as much blood as that required by the D.C.A. treated dog. Arteriolar capacity adjustments appeared to be lacking in the animals lacking hormone reserves.

Following completion of the hemorrhage, the intact animals, the adrenalectomized dogs given fore-treatment with D.C.A., and those receiving large amounts of cortical extract, all showed full recovery of the arterial pressure to normal levels with disappearance of all symptoms. Two of the four D.C.A. treated dogs recovered within 7 hours following the bleeding. The other two animals exhibited an immediate pressure rise of 22 to 24 mm. Hg and then suffered a partial relapse for 4 to 5 hours, later recovering completely in 36 to 48 hours without additional treatment of any kind.

Dogs receiving merely maintenance doses of cortical extract may show,

on cessation of the bleeding, a spontaneous rise of blood pressure varying from 10 to 20 mm. Hg. However, the rise was not sustained and a regression invariably followed, leading to death from circulatory collapse

TABLE 1

Blood studies of adrenalectomized dogs subjected to hemorrhage

DATE	TIME	BLOOD PRESSURE	PULSE	HEMATOCRIT	HEMOGLOBIN	SERUM SODIUM	SERUM CHLORIDE	SERUM POTASSIUM	BLOOD UREA NITROGEN	BLOOD SUGAR	REMARKS
Dog 1. 9.0 kgm. 20 mgm. D.C.A. Removed 33.3 cc./kgm. body weight blood											
		mm. Hg	*per minute*	*per cent*	*grams per cent*	*m-eq. per l.*	*m-eq. per l.*	*m-eq. per l.*	*mgm. per cent*	*mgm. per cent*	
1/9	10:30 a.m.	110	84	41.8	12.6	143.5	112.2	4.4	9.3	83	
	10:52 a.m.	45	88	36.1	10.6						Completed bleeding. Weak, swaying gait
	2:00 p.m.	92	148	30.6	9.9						Refused water, appears normal
	8:30 p.m.	95	108	28.5	8.3	142.5	113.8	8.0	17.0	83	Ate full ration, drank water
1/10	10:00 a.m.	112	116	28.7	8.4						Normal
Dog 2. 13.2 kgm. 20 mgm. D.C.A. Removed 29.3 cc./kgm. body weight blood											
1/23	10:00 a.m.	106	48	41.8	11.9	142.7	109.4	5.1	18.3	79	
	11:30 a.m.	46	96	41.5	11.8						Completed bleeding, weak, listless
	2:00 p.m.	70	108	30.2	7.7						Stronger
	5:00 p.m.	57	108								Partial relapse, inactive
	8:00 p.m.	75	108	27.4	7.2	140.6	107.2	4.6	18.5	83	Alert, active, ate full ration
1/24	9:30 a.m.	88	72	24.1	6.2						Appears normal
1/25	9:30 a.m.	104	68	24.5	6.5	143.2	111.0	4.6	17.3	84	Normal
Dog 3. 10.2 kgm. 3 cc. cortical extract daily. Removed 17.3 cc./kgm. body weight blood											
3/4	9:35 a.m.	110	70	41.6	13.6	143.4	113.2	4.8	27.0	83	
	10:45 a.m.	47	72	36.0	11.8						Completed bleeding. Weak
	1:50 p.m.	75	96	30.7	10.8						Strong but listless
	10:30 p.m.	60	96	27.1	9.6	142.5	107.0	5.9	69.0	80	Refused food, alert
3/5	9:30 a.m.	46	124	30.5	10.0						In collapse. Revived with cortical extract
Dog 4. 8.0 kgm. Cortical extract withdrawn for 24 hours. Removed 15.9 cc./kgm. body weight blood											
3/11	11:00 a.m.	106	100	36.7	12.7	141.4	108.0	5.7	35.6	81	
	11:30 a.m.	44	88	32.8	12.2						Completed bleeding
	2:30 p.m.	51	156	33.4	12.5						Weak, lethargic
	4:30 p.m.	38	148	34.8	12.8	137.3	111.2	7.6	62.5	90	Died ½ hour later

within 8 to 20 hours. Animals deprived of extract for 24 hours previous to hemorrhage usually did not exhibit even this temporary rise in pressure, and the circulation failed within a shorter interval.

The D.C.A. primed dogs rapidly diluted their blood following hemorrhage, just as does the animal with intact adrenals (table 1). The hemo-

dilution observed in the animals bled while receiving maintenance doses of extract was, however, variable in extent. One dog showed a dilution equivalent to that characteristic of D.C.A treated animals (table 1, dog 3). It is of interest to note that the blood pressure of this dog was but temporarily elevated above shock levels even though the restoration of blood volume, as evidenced by hemodilution values, was of similar degree to that of the intact or D.C.A. treated animals. (Compare dog 3 with dogs 1–2, table 1.)

The animals not receiving extract for 24 hours prior to hemorrhage showed little if any signs of hemodilution at any time during the experiments.

Changes in blood chemistry characteristic of adrenal insufficiency were not found in any of the dogs subjected to hemorrhage. The blood glucose and serum chloride levels usually remained unchanged. The serum sodium concentration was usually slightly lowered, and serum potassium was elevated in some cases but not in others. A small increase in blood urea nitrogen was occasionally noted. The changes observed were small in magnitude and not always present. The data presented in table 1 indicate that blood glucose and serum electrolyte changes obviously can not be regarded as important factors in the circulatory failure resulting from hemorrhage in these adrenalectomized dogs.

II. *Ineffectiveness of D.C.A. in preventing circulatory collapse following the surgical trauma incident to a single stage bilateral adrenalectomy.* Removal of both adrenal glands at a single stage operation in the dog is almost invariably followed by death within 10 to 24 hours. Large doses of cortical extract will restore the prostrate animal to normal health and vigor; moreover, foretreatment of the animal with large amounts of extract will protect against the circulatory failure (12). However, priming the animals with D.C.A. has, in our experience, proven quite ineffectual in prolonging the survival period (13) (table 2).

The underlying basis for the rapidly fatal outcome of this type of operation is not clear. It is well established that the single stage bilateral adrenalectomy does not induce circulatory failure in such species as the rat and cat. The collapse can not be attributed to alterations in serum electrolyte pattern, hemoconcentration, and loss of body water, changes which are usually associated with adrenal insufficiency. As shown in table 3, all changes in the blood chemistry in the terminal stages of the circulatory failure following the single stage bilateral operation, are negligible, with the exception of a decline in blood sugar and this is by no means invariable. An occasional animal may also show a sharp rise in serum potassium, e.g., dog 13, table 3.

The fall in glucose may or may not attain hypoglycemic levels. It might seem that the fall in blood pressure was directly related to this decline in blood sugar. However, some animals show normal sugar values

with arterial pressures at shock levels (table 2). Moreover, there is no clear correlation between the blood sugar level and the onset of circulatory

TABLE 2

Blood pressure and blood sugar changes following single stage bilateral adrenalectomy in the dog

DOG	INITIAL		5 HOURS		9 HOURS		12 HOURS		24 HOURS		48 HOURS	
	Blood pressure	Blood sugar	Blood pressure	Blood sugar	Blood pressure	Blood sugar	Blood pressure	Blood sugar	Blood pressure	Blood sugar	Blood pressure	Blood sugar
Untreated animals												
	mm. Hg	*mgm. per cent*	*mm. Hg*	*mgm. per cent*	*mm. Hg*	*mgm. per cent*						
5	114	87	77	82	64	82	Revived with cortical extract					
6	106	82			42	64	Died at 12 hours					
7	104	84			43	44	Revived with cortical extract					
8	110	87	95	80	63	70	Died at 15 hours					
Ave.	108	85	86	81	53	65						
Animals receiving 20 mgm. D.C.A. before operation												
9	106	89	105	83	54	68	Revived with cortical extract					
10	116	84	114		42	64	Died at 13 hours					
11	110	87	101		55	54	Revived with cortical extract					
12	114	83	64	76	37	40	Died at 10 hours					
Ave.	111	86	96	80	47	57						
Animals receiving 20 mgm. D.C.A. and intramuscular glucose												
							mm. Hg	*mgm. per cent*	*mm. Hg*	*mgm. per cent*	*mm. Hg*	*mgm. per cent*
13	112	83	112	89	82	91	66	91	45	77	62	111
14	110	88	108	87	73	92	66	56	50	50	73	79
Ave.*	111	86	110	88	78	91	66	74	48	64	68	95
Animals operated with novocaine infiltration. 1–2 mgm. D.C.A./day												
15	108	88	115	65	88	85	88		96	87	112	87
16	110	84	112	83	99	71	99		112	80	110	85
17	110	88	116	86	106	64	106		106	87	110	88
18	112	77	116		106	65	106		110	75	114	75
Ave.†	110	84	115	78	100	72	100		106	82	112	84

* Animals sacrificed at 72 hours (see text).

† All animals survived on maintenance levels of D.C.A.

failure. To test this possible relationship between blood pressure and sugar further, two dogs were bilaterally adrenalectomized at a single stage operation in the usual way, and then hourly intramuscular injections of 10 cc.

of a 10 per cent glucose solution were given for the first twelve hours after the operation. The injections were intended to maintain the blood sugar at normal levels or above. The blood pressure decline in these two dogs was far less severe and slower than we had previously observed (table 2).

TABLE 3

Blood studies on dogs subjected to single stage bilateral adrenalectomy

DATE	TIME	BLOOD PRESSURE	PULSE	HEMATOCRIT	HEMOGLOBIN	SERUM SODIUM	SERUM CHLORIDE	SERUM POTASSIUM	BLOOD UREA NITROGEN	BLOOD SUGAR	REMARKS
		Dog 9. 7.1 kgm. 20 mgm. D.C.A.									
		mm. Hg	*per minute*	*per cent*	*grams per cent*	*m-eq. per l.*	*m-eq. per l.*	*m-eq. per l.*	*mgm. per cent*	*mgm. per cent*	
12/2	11:00 a.m.	106	80	44.0	13.5	144.3	111.4	6.0	22.1	89	
12/3	12:15 p.m.	108	188							83	Completed operation
	9:15 p.m.	54	140	44.5	14.0	141.3	110.0	5.0	25.4	68	Near collapse, cortical extract injected
12/4	11:30 a.m.	96	100							84	Appears normal
		Dog 11. 8.6 kgm. 20 mgm. D.C.A.									
12/6	10:00 a.m.	110	90	47.5	14.9	145.8	111.0	4.5	15.3	87	Completed operation at 11:00 a.m.
	8:00 p.m.	55	152	50.2	16.3	143.0	109.0	6.2	20.2	54	Prostrate. Died at 11:00 p.m.
		Dog 13. 8.5 kgm. 20 mgm. D.C.A. Intramuscular glucose									
12/13	10:00 a.m.	112	68	42.8	14.4	143.8	111.6	6.8	15.0	83	Completed operation at 11:15 a.m.
12/14	1:00 a.m.	66	168	43.2	16.4	143.1	109.0	10.2	18.2	83	Weak, cannot walk
	10:30 a.m.	45	176	46.3	17.0	145.8	109.6	8.9	20.0	77	Weak, sacrificed at 72 hours
		Dog 17. 10.2 kgm. Novocaine infiltration. 2 mgm. D.C.A./day									
2/12	10:00 a.m.	110	72	42.3	12.5	143.6	111.8	7.0	24.6	88	Completed operation at 11:55 a.m.
	9:00 p.m.	106	144	44.4	13.4	144.0	112.0	6.7	30.5	64	Active, ate full ration, normal
		Dog 18. 10.0 kgm. Novocaine infiltration. 1 mgm. D.C.A./day									
2/25	11:00 a.m.	112	90	47.9	16.0	142.5	110.0	5.8	23.9	77	Completed operation at 1:00 p.m.
	11:00 p.m.	106	196	42.8	15.4	142.0	110.7	4.5	20.4	65	Strong, alert

Shock pressure levels were not reached until 20 to 24 hours after the operation. One of the two animals developed a low blood sugar at this time. Glucose injections were then continued at frequent intervals, thereby maintaining the blood sugar level normal or above for the rest of the experiment. Despite the treatment the animals remained extremely lethar-

gic, often semi-comatose, would not stand or walk, refused all food, and were sacrificed at 72 hours. The blood pressures showed some elevation from the 24 hour level, but never approached the normal. Hence, although the extra glucose definitely prolonged the survival period, the blood pressure remained low and the fatal outcome was merely postponed, the animals dying with normal, or near normal levels of blood sugar. Although it is evident that these animals did not die of hypoglycemia, disturbances in carbohydrate metabolism apparently are associated with this type of shock inducing procedure.

III. *Effectiveness of D.C.A. in preventing circulatory failure following a single stage bilateral adrenalectomy when the nerves in the vicinity of the adrenals are blocked by procaine.* Freud *et al.* (14) and others have called attention to the presence of sympathetic plexuses in the immediate vicinity of the adrenal glands of dogs, and have emphasized the fact that, despite careful surgical technique, considerable injury and trauma to these nerves is unavoidable when the glands are removed. These authors attribute some of the symptoms generally regarded as characteristic of acute adrenal insufficiency, to injury of the nerves in the proximity of the glands.

Firor has routinely adrenalectomized dogs at a single stage operation using *spinal anesthesia* and Thorn (1) states that the animals so operated can be maintained on D.C.A. In order to test the extent to which nerve trauma was influencing the effectiveness of D.C.A. priming in our experiments, six dogs were prepared for the single stage operation in the usual manner. Before dissecting the glands previous to removal, and during the whole of the operation, the adrenals and surrounding tissues were thoroughly bathed and infiltrated with a 4 per cent novococaine solution. Approximately 10 cc. of the solution were used around each gland (tables 2 and 3). Upon recovery from the general anesthetic the dogs were alert, active and eager for food. A full ration was taken the day of operation. The blood sugar of these animals, however, still showed some decline from normal until food was taken, when it was promptly restored. No signs of circulatory failure appeared and the blood pressure fall was not over 20 mm. Hg (table 3). Whereas 20 mgm. D.C.A. used as a prophylactic foretreatment had failed to protect the dog bilaterally adrenalectomized under a general anesthetic only, the animals in which the nerve plexuses around the glands had been locally blocked could be easily maintained in excellent condition by 1 to 2 mgm. D.C.A. per day.

Discussion. The response to hemorrhage of the adrenalectomized dog primed with D.C.A. differs from that of the untreated animal in four major respects: 1. Approximately twice as much blood can be removed before the blood pressure falls to shock levels. 2. Throughout the major part of the hemorrhage, the pressure remains normal or above, while in the animal not receiving extract the pressure drops gradually but continuously from the

time of first withdrawal of blood. 3. Both during and following bleeding, the blood is rapidly diluted from extra-vascular sources in the D.C.A. primed dog whereas the untreated animal shows no blood dilution. 4. The arterial pressure spontaneously returns to normal in the primed dog, but the animal lacking hormone reserves exhibits little or no elevation of pressure from shock levels.

The circulation of the non-primed animal is apparently deficient in two important respects: 1. In the absence of hormone the dog seems unable to make adequate compensatory capacity adjustments of the arterioles. 2. He is unable to dilute the blood by withdrawal of tissue fluid into the capillaries. Both of these deficiencies are corrected by injection of extract or prevented from appearing by foretreatment with either D.C.A. or extract. The evidence suggests that the circulatory deficiencies of adrenal cortical insufficiency, as well as those which appear as a result of hemorrhage in the dog lacking adrenals, are due primarily to 1, atony of the arterioles with resulting inability to sustain a prolonged vasoconstriction; 2, atony of the capillaries with consequent pooling and stagnation of blood, anoxia and increased permeability. These changes render ineffectual the normal mechanism for fluid exchange.

It is significant that priming foretreatment of adrenalectomized dogs with D.C.A. is useless as a preventive against circulatory failure induced primarily by injury to the nervous system in the splanchnic area. We have previously called attention to the ineffectiveness of D.C.A. in preventing circulatory collapse which follows intestinal manipulation (4). Both intestinal stripping and single stage bilateral adrenalectomy are shock inducing procedures belonging in the same category since both involve extensive trauma to nervous elements in the visceral region, and both are unresponsive to D.C.A. Corticosterone, in contrast to D.C.A., will afford protection to the circulation in intestinal trauma and may also, when employed as a prophylactic, afford protection against the circulatory collapse following the single stage bilateral adrenalectomy.

Since local blocking of the nerves or spinal anesthesia effectively prevented circulatory failure from developing after the single stage operation, it is evident that afferent nerve impulses originating in the traumatized region must play an essential rôle in the etiology of the circulatory collapse resulting from this type of surgical trauma in the adrenalectomized dog.

In so far as the dog lacking adrenals is concerned, D.C.A. is an effective prophylactic only for those types of shock which are essentially non-nervous in origin.

SUMMARY

1. Desoxycorticosterone acetate, when used as a prophylactic foretreatment, protects the adrenalectomized dog against circulatory failure following hemorrhage.

2. The chief circulatory disabilities of the dog lacking adrenals and subjected to hemorrhage are: 1, inability to sustain a prolonged vasoconstriction, and 2, inability to dilute the blood. D.C.A. or extract corrects these deficiencies, or prevents their appearance when used as prophylactic foretreatment.

3. D.C.A., unlike cortical extract, is noneffective in preventing the circulatory collapse following a single stage bilateral adrenalectomy in the dog.

4. Local blocking of the nervous elements in the proximity of the adrenals before their removal prevents the onset of circulatory failure. Animals so treated can be maintained on small doses of D.C.A.

5. Circulatory failure following the single stage bilateral operation in the dog is therefore apparently due to afferent nervous impulses originating in the injured area.

6. In our experience, D.C.A. affords no protection to the circulation of the adrenalectomized dog, in those types of shock due primarily to injury and trauma to nervous elements in the splanchnic area.

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TERMINAL CEREBROSPINAL FLUID PRESSURE VALUES IN VITAMIN A DEFICIENCY[1]

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In a previous paper Moore and Sykes (1940) reported that a deficiency of vitamin A in the ration of calves produced an increased cerebrospinal fluid pressure accompanied by papilledema, nyctalopia, syncope and incoördination. The addition of crystalline carotene to the ration caused these disturbances to disappear and the cerebrospinal fluid pressure gradually returned toward normal. In the previous work the cerebrospinal fluid pressure was not permitted to increase markedly since it was desired to restore the pressure to normal levels as quickly as possibly by carotene therapy. It is the purpose of the present paper to report values for the pressure of the cerebrospinal fluid where the animals were permitted to proceed to the terminal stage of vitamin A deficiency.

Experimental. The animals used in this experiment had been maintained on various levels of carotene to determine the amount of carotene required to prevent the development of an increased cerebrospinal fluid pressure in the growing calf. In the experiments reported here the source of carotene was removed from the ration of these animals in order to allow the various individuals to develop severe vitamin A deficiency symptoms.

The low carotene ration consisted of skimmed milk and a concentrate mixture consisting of 240 pounds barley, 180 pounds rolled oats, 180 pounds wheat bran, 60 pounds linseed oil meal and 8 pounds salt. This ration contained sufficient carotene to supply two to four micrograms per kilogram of body weight per day. Wood shavings were used as bedding.

Blood plasma carotene determinations were made each week according to the method of Moore (1939). Cerebrospinal fluid pressures were obtained by puncture into the subarachnoid space. The insertion was made through the dorsal opening in the atlanto-occipital articulation. No anesthetic was used and the records were obtained with the animals standing quietly.

Animal I was an Ayrshire female which since four months of age had been receiving carotene from alfalfa leaf meal at a rate of 60 micrograms of

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carotene per kilo of body weight. At this intake of carotene the cerebrospinal fluid pressure increased to about double the normal value and some slight swelling of the nerve head was observed. Otherwise the animal appeared normal. At 498 days of age the alfalfa meal was removed from the ration and at 511 days a convulsive seizure was noted. Convulsions were seen periodically during the succeeding period and could sometimes be brought on by excitement caused by such procedures as drawing blood samples. During the last two weeks this animal moved around very little and had some difficulty getting up. Diarrhea developed and a marked

TABLE 1

Terminal cerebrospinal fluid pressure values in vitamin A deficiency

AGE	PLASMA CAROTENE	CEREBROSPINAL FLUID PRESSURE	AGE	PLASMA CAROTENE	CEREBROSPINAL FLUID PRESSURE
Animal I			Animal III		
days	*micrograms per ml.*	*mm. of saline*	*days*	*micrograms per ml.*	*mm. of saline*
431	1.0	190	330	0.35	125
458	1.0	190	378	0.35	120
501	0.7	205	398	0.35	140
536	0.2	345	428	0.25	170
563	0.12	380	566	0.10	360
594	0.01	520	583	0.10	580
605	0.01	600	604	0.03	400
612	0.02	460	640	0.02	310
Animal II			Animal IV		
726	0.7	110	431	0.81	220
755	0.2	125	466	0.95	300
825	0.05	280	500	0.87	210
831	0.05	470	535	0.19	290
			563	0.07	420

papilledema was present. At 620 days of age the animal was unable to rise and was sacrificed in order to save the tissue for pathological study. The principal results are shown in table 1 from which it will be noted that as the plasma carotene dropped, the cerebrospinal fluid pressure increased and reached a maximum of 600 mm. of saline. A week later the pressure dropped to 460 mm.

Animal II was a Holstein male which had been receiving 120 micrograms of crystalline carotene dissolved in cottonseed oil per kilo of body weight. The carotene was removed from the ration at 726 days of age after which incoördination and convulsions were apparent at 771 days. Papilledema was evident at 801 days, the animal was unable to rise at 831 days of age

and was sacrificed at that time. The principal results are shown in table 1. It will be noted here that the cerebrospinal fluid pressure reached a maximum of 470 mm. after carotene was removed from the ration. Animal III was also a Holstein male which had been receiving 60 micrograms of carotene per kilo of body weight. The alfalfa was removed from the ration at 394 days of age. Papilledema and incoördination developed at 566 days and the animal died at 648 days. The principal results are shown in table 1. It will be noted that this animal like animal I showed a terminal drop in cerebrospinal fluid pressure after developing a maximum pressure of 580 mm.

Animal IV was an Ayrshire male which had been receiving 45 micrograms of carotene per kilo of body weight and had developed an elevated pressure at this level of carotene intake. The alfalfa was taken out of the ration at 498 days of age after which the animal showed marked papilledema and incoördination at 563 days. It was necessary to sacrifice the animal at 571 days of age. When carotene was withdrawn from the ration the pressure increased to 420 mm.

It was possible to increase the high pressure of these animals still further by excitement such as slapping them on the back. This excitement often caused approximately a two-fold increase. In one instance, an increase to 1060 mm. from a previous level of 460 mm. was observed and in another a pressure of 560 mm. developed during excitement from a previous level of 290 mm.

Discussion. The results show that when young bovine are placed on a low carotene ration the cerebrospinal fluid pressure may attain values from 400 to 600 mm. of saline. This would be about 4 to 6 times the normal value. These values were obtained with animals at the terminal stage of vitamin A deficiency. In two cases there was a terminal drop in pressure from a previously higher level. Usually the animals in this condition had very little appetite, showed diarrhea and were more or less in a moribund state so that the drop was not surprising.

The increase in cerebrospinal fluid pressure was always accompanied by a marked papilledema, incoördination and periods of syncope. The condition of syncope often proceeded to a state of convulsive seizure during which respiration ceased for short periods of time. It was felt that the syncope and convulsive like seizures were due to a cerebral anemia because of the increased cerebrospinal fluid pressure. The fact that excitement often caused these seizures further indicates that this may be true since it has been shown that a marked increase in pressure accompanies periods of disturbance.

The particular cause of the increased cerebrospinal fluid pressure in vitamin A deficiency in the bovine has not been found. Pathological study of the choroidal plexus and arachnoid villi, colloidal osmotic pressure measure-

ments of blood plasma and various blood and urine analysis have not shown any abnormality which could be related to the raised pressure. In this connection, however, Wolbach and Bessey (1940) have reported a relative overgrowth of the central nervous system in vitamin A deficiency in rats. They report extensive and striking herniations of the nerve roots into the intervertebral foramina, herniations into the bodies of the vertebrae, an increase in size of the contents of the cranium as evidenced by *a*, the presence of herniations of the cerebrum, cerebellum and posterior colliculus; *b*, distortion of the brain; *c*, changes in the contours of the fossae of the floor of the skull. If there is a relative overgrowth of the central nervous system in the bovine such as reported by Wolbach and Bessey in rats it could easily account for the increased cerebrospinal fluid pressure reported here. Evidence of bony changes in the cranial cavity in vitamin A deficiency in calves has already been reported by Moore, Huffman and Duncan (1935) and Moore (1939) in which a blindness was produced by a stenosis of the bony optic canal through which the optic nerve passes. In the previous paper it was reasoned that the bony malformations in calves were possibly due to the increased cerebrospinal fluid pressure.

Wolbach and Bessey (1940) have expressed belief that the relative overgrowth of the central nervous system is a growth phenomenon. However, Moore (1941) has shown that papilledema due to vitamin A deficiency will develop in mature cows which might indicate that the increase in the volume of the central nervous system was not entirely confined to the growth period. De Schweinitz and De Long (1934) have presented evidence that a perivascular edema of the brain tissue accompanies papilledema and blindness in calves which may indicate that a similar increase in volume of the cranial contents is the cause of the raised pressures observed in vitamin A deficiency. It seems likely that the increased pressure is due to an increased volume of the cranial contents caused either by a relative overgrowth as indicated by Bessey and Wolbach or to an actual increase in the volume of the cranial contents.

SUMMARY

1. Terminal cerebrospinal fluid pressure values of 400 to 600 mm. of saline were recorded in young bovine fed a vitamin A deficient ration.

2. The possible cause of the increased pressure is discussed.

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SOME FACTORS IN SECRETION BY SUBMAXILLARY GLANDS OF CATS

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The work to be reported here was undertaken to study the effects of various frequencies of stimulation of the chorda tympani upon the secretory response of the submaxillary gland, and to investigate some of the factors in the functioning of the gland.

Experimental. Cats anesthetised with Dial were used in all experiments. The submaxillary duct was cannulated with a capillary glass tube, and salivary flow was registered by a drop counter similar to that of Gesell (1929). The chorda tympani was dissected out in the neck and stimulated through platinum electrodes by a thyratron stimulator. Stimulation with pilocarpine was achieved either by intermittent injection into a femoral vein or by continuous injection into the isolated arterial supply of the submaxillary gland. Blood flows were obtained by isolating the return from the stimulated gland and registering the venous flow with another drop counter, the blood of the animal having been rendered non-coagulable by intravenous injection of "Liquoid" (Hoffman-LaRoche). Potassium was determined by the method used by Fenn et al. (1938).

The effects of various frequencies of stimulation of the chorda tympani upon the secretory response of the submaxillary gland are shown in figure 1. This figure presents the response of a single submaxillary gland during each minute of the first five minutes of stimulation at various frequencies but with constant strength of stimulus. The curves drawn here are typical of those obtained in five such experiments. These graphs show that the salivary secretion during the first minute of stimulation was practically constant for a fairly wide range of frequencies. However, at a frequency of 60 per sec. the initial secretion was small. The optimal rate of stimulation appeared to be about 9 per sec. At this frequency the

initial high secretory output was best maintained. Essentially the same conclusions can be reached from a consideration of figures 2 and 3, although there the picture is somewhat complicated by the fact that each curve represents a separate animal.

At first these findings seem to conflict with those of Rosenblueth (1932), who reported that the optimal frequency for stimulation of the chorda tympani was 36 shocks per sec. Rosenblueth's experiments lasted for

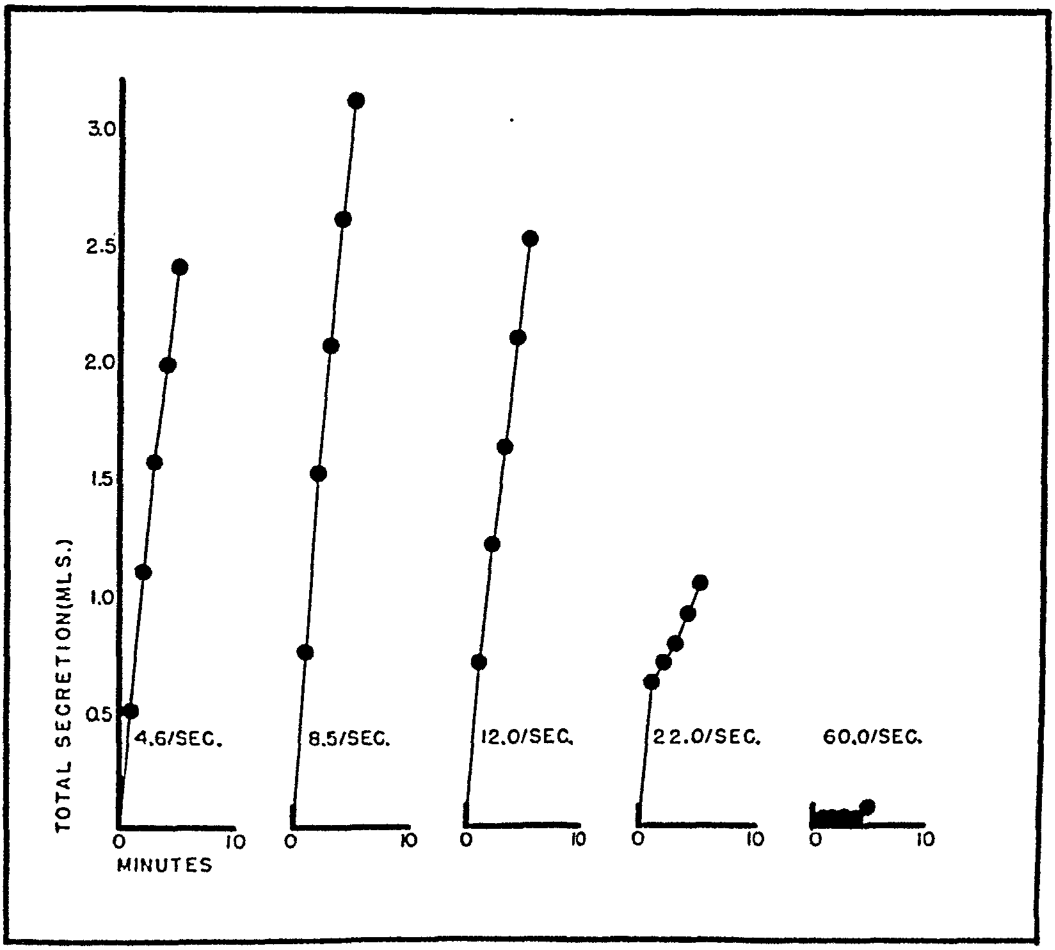

Fig. 1. Responses of a single submaxillary gland during each minute of the first five minutes of electrical stimulation of the chorda at various frequencies but with constant strength of stimulus.

only 30 seconds; there is evidence in his paper that if stimulation had been prolonged, a lower value for the optimal frequency would have been obtained. By reference to figure 7 of Rosenblueth's paper it can be seen that while frequencies of stimulation of around 25 per sec. produced greater initial rates of secretion than did those of around 9 per sec., the primary rate was better maintained at the lower frequency even for the half-minute duration of those experiments. Frequencies of stimulation

below about 9 per sec. produced well-maintained rates of secretion, but these rates were below those produced by the 9 per sec. frequency. Therefore it seems likely that there is no real disagreement between Rosenblueth's work and the present experiments.

Figure 2 presents the results of nine experiments in which electrical stimulation of the chorda tympani at various frequencies was continued for about three hours, the strength of stimulus in any one experiment being constant throughout. The fact that each curve represents a different

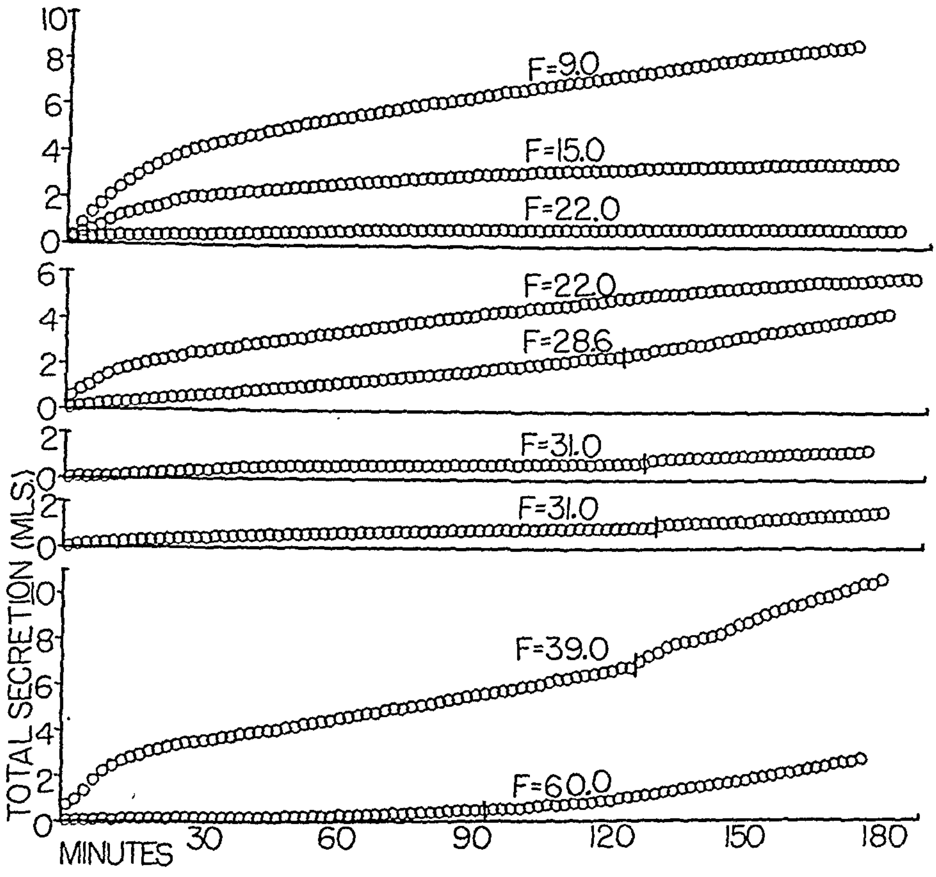

Fig. 2. Variations with time of the total amount of saliva produced by submaxillary glands stimulated for long periods at various frequencies.

animal probably explains in part the different amounts of total secretion obtained in the various experiments. However, the shape of the curves is of more interest at present than their magnitude.

It will be seen that some of the graphs of figure 2 have a vertical line drawn on them. This line indicates what seems to be a fairly sudden increase in the rate of secretion, occurring between the 123rd and 131st minute after commencement of stimulation at frequencies between 28.6 and 39.0 per sec. In the experiment with stimulation at 60.0 per sec. this sudden increased rate of salivation appeared at the 94th minute. The

experiments at frequencies below 28.6 shocks per sec. showed no sign of such an increase, even though one of the experiments at a stimulation rate of 22.0 per sec. was prolonged to the 218th minute after commencement of stimulation.

The sigmoidal first portions of the curves of figure 2 suggest that careful analysis of this section of these graphs would reveal a rapid, a slow and again a rapid rate of salivary production. To test this supposition, the rates of secretion during each of the first twenty minutes for some of the experiments have been plotted in figure 3. Here it appears that in every case there was an initial period of a relatively high rate of secretion, followed by a falling off. In the experiments at frequencies of 39.0 or less shocks per sec. there was a secondary increase in rate at times varying from the sixth to the twelfth minute of stimulation. During this period the rate of secretion might increase to as much as 40 per cent of the rate during the first minute. Then the speed of secretion decreased again and leveled off into the continuous and uniform output making up the major portion of the curves of figure 2.

These changes in rate of secretion show a certain similarity to the changes of tension of muscles during long-continued stimulation (Rosenblueth and Morison, 1937) (Rosenblueth and Luco, 1939). In particular, the late increase in rate of saliva production shows similarity to Rosenblueth's "fifth stage" in muscle by beginning earlier with higher frequencies of stimulation.

By referring again to figure 3 it will be seen that the experiment with stimulation at a frequency of 60 shocks per sec. had only fleetingly a relatively high rate of secretion, salivary production falling to a uniform low level within two minutes of commencement of stimulation. By following the interpretation of a similar situation in muscle by Rosenblueth and Luco (1939), it could be said that there was absence of stage two with merging of stages one and three. This latter combined phase was completed in the first minute or so, and stage four then began and lasted until the beginning of stage five at the 94th minute.

Another series of experiments was undertaken to study simultaneous variations in blood and salivary flows with both chorda and pilocarpine stimulations. The submaxillary glands of seven animals were stimulated by electrical excitation of the chorda tympani, at frequencies ranging from 7.9 to 31.0 shocks per sec.; there were five animals with pilocarpine as stimulant of the gland.

Table 1 presents the averaged data from these experiments together with other similar averages collected from the literature. It will be noted that the figures for resting blood flow in the cats are markedly lower than those quoted for dogs. This difference can be very largely removed by calculating the figures on the basis of the weight of the submaxillary gland.

When this is done, it is found that the cat had an average resting blood flow through its submaxillary gland of 0.20 ml. per min. per gram of tissue; from the data of Anochin, Goldberg and Samarina (1930) the dog had a resting blood flow of 0.25 ml. per min. per gram of tissue, and from the data of Tatibana (1936–38) it had one of 0.27 ml. per min. per gram of gland.

The figures of table 1 show that with secretion by the submaxillary gland there was an increased blood flow through the gland whatever the stimulant. However, they also seem to show that the amount of saliva produced with a given blood flow was greater with pilocarpine stimulation

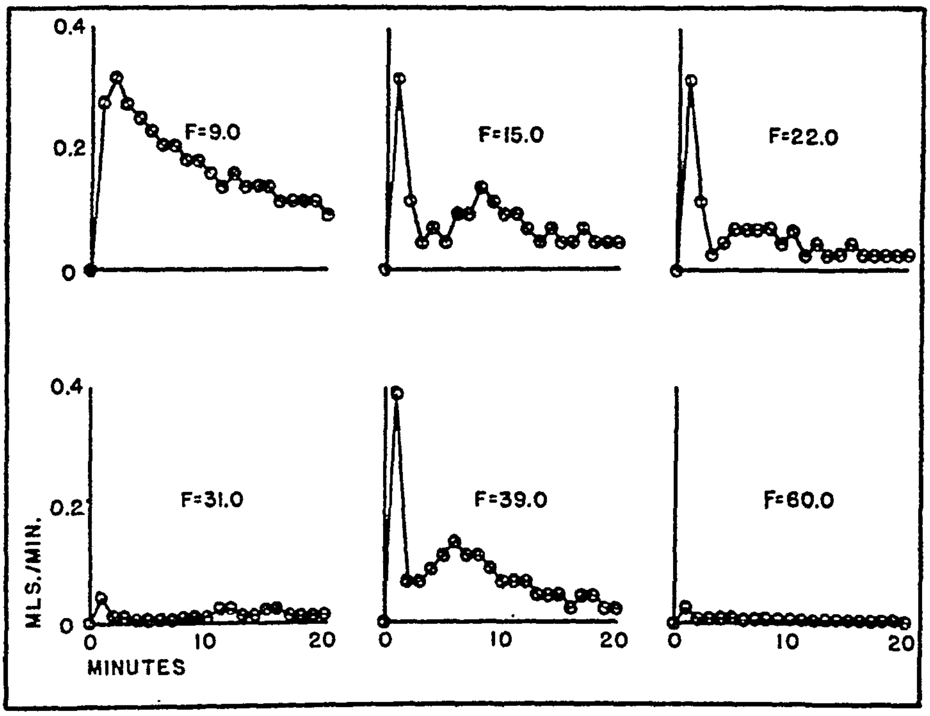

Fig. 3. Variations with time of the rate of saliva production by submaxillary glands stimulated at various frequencies.

than with chorda excitation. The latter point will be discussed further in a subsequent portion of this paper.

From the data of Barcroft, Barcroft and Kato and Tatibana in table 1, it can be calculated that the assumption that arterial blood flow equals venous blood flow plus saliva flow yields values for arterial flow low by only 2.3 per cent of the correct figure. Therefore, in the experiments reported here arterial blood flows during activity were calculated by summing venous and saliva flows. Basal arterial flows were assumed to be the same as basal venous flows, an assumption supported by the data of Barcroft and Kato and of Tatibana. The superbasal arterial flow to the submaxillary gland during activity could now be calculated.

It was found that in 75 cases of electrical excitation of the chorda for periods of about ten minutes the ratio of superbasal blood flow to saliva flow had an average value of 12.6 with a standard deviation of the mean of $\pm$ 1.1; in 21 similar periods with pilocarpine as stimulant the value of the ratio was 4.5 $\pm$ 0.5. In figure 4 the superbasal arterial flow has been plotted on the abscissa against the saliva flow on the ordinate for experiments with electrical excitation of the chorda and with pilocarpine stimulation. On the same graph have been drawn straight lines with slopes

TABLE 1

Blood and saliva flows of submaxillary glands with chorda and pilocarpine stimulations

AUTHOR	ANIMAL	STIMULANT	RESTING		STIMULATED		
			Arterial blood flow	Venous blood flow	Arterial blood flow	Venous blood flow	Saliva flow
			ml./min.	*ml./min.*	*ml./min.*	*ml./min.*	*ml./min.*
Barcroft, 1900	Dog	Chorda			13.2	12.0	1.0
Anrep and Cannan, 1922	Dog	Chorda		1.44		3.88	0.4
Anochin, Goldberg and Samarina, 1930	Dog	Chorda		1.16		3.02	
McClanahan and Amberson, 1935	Cat	Chorda				3.82	0.16
Tatibana, 1936–38	Dog	Chorda	1.81	1.81	14.26	12.70	0.95
Present author	Cat	Chorda		0.22*		3.77‡	0.25¶
Barcroft and Kato, 1916	Dog	Pilo.	1.40	1.40	4.66	4.20	0.36
McClanahan and Amberson, 1935	Cat	Pilo.				1.43	0.27
Tatibana, 1936–38	Dog	Pilo.	1.33	1.33	4.61	3.93	0.46
Present author	Cat	Pilo.		0.22†		4.78§	1.11‖

* Average of 82 determinations, with standard deviation of the mean of $\pm$ 0.01.
† Average of 18 determinations; $\pm$ 0.01.
‡ Average of 75 determinations; $\pm$ 0.38.
§ Average of 21 determinations; $\pm$ 0.75.
¶ Average of 75 determinations; $\pm$ 0.04.
‖ Average of 21 determinations; $\pm$ 0.18.

calculated from the average values of the blood to saliva flow ratios. These lines can be seen to fit the two groups of points reasonably well, indicating in both cases a direct proportionality between extra blood flow and saliva production as found by Gesell (1919) in the case of chorda stimulation. The fact that the line for pilocarpine stimulation is higher than that for chorda excitation furnishes proof that pilocarpine stimulation of the submaxillary gland of cats produced a greater saliva flow for a given blood flow than did electrical stimulation.

From table 1 it is possible to calculate values of the ratio of mean superbasal blood flow to mean saliva flow for the dog in some cases. Tatibana's

data yield values for this ratio of 13.1 with chorda stimulation and of 7.1 with pilocarpine. The data of Anrep and Cannan yield a value of 7.1 for the blood to saliva flow ratio with chorda stimulation, while from the figures of Barcroft and Kato comes a value of 9.1 with pilocarpine. Since Anrep and Cannan say in their paper that they tried to keep blood flow low, it is possible that their data are not representative. It seems safe to conclude, therefore, that in both dog and cat pilocarpine stimulation of the submaxillary gland produced a greater secretion of saliva for a given blood flow than did chorda stimulation.

The higher saliva production with a given blood flow after pilocarpine injection may account in part for the effect of pilocarpine on the potassium content of the submaxillary gland (Wills and Fenn, 1938). It was found that as the output of potassium in the saliva increased the submaxillary

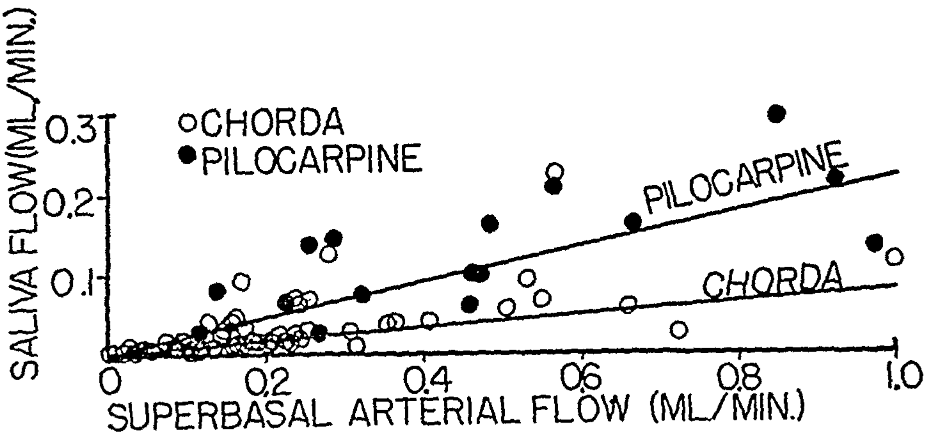

Fig. 4. Changes in the rate of saliva secretion with changes in the rate of superbasal arterial flow for submaxillary glands stimulated through the chorda or with pilocarpine.

gland was able to keep its potassium content normal if the stimulation was effected through the chorda tympani, while with pilocarpine stimulation the potassium concentration in the gland decreased rather markedly. Certainly such a difference between these two types of stimulation would be expected to show up if the potassium supply from the blood in the former case were potentially several times greater than in the latter, as now seems evident.

Langstroth, McRae and Stavraky (1938) have reported that, with electrical stimulation, at rates of saliva flow below 0.17 ml. per min. the concentration of potassium in the saliva increased with decreasing rate. At greater velocities of flow the potassium concentration was constant. A number of values for saliva potassium from the foregoing experiments are plotted against the rate of secretion in figure 5, and will be seen to conform roughly to the curve of Langstroth, McRae and Stavraky. How-

ever, it seems that the point of inflection comes at about 0.03 ml. per min. rather than at 0.17. The data of the other authors plotted on the same graph (as solid squares) seem to fit this curve almost as well as they do that in the original paper. The saliva secreted after pilocarpine stimulation apparently followed the same rule as that produced by chorda stimulation, so far as can be judged from the few data available. From this graph it can be seen also that at very low rates of secretion there was an apparently greater output of potassium by the submaxillary gland than might have been expected from the rate of secretion. This might mean that at these

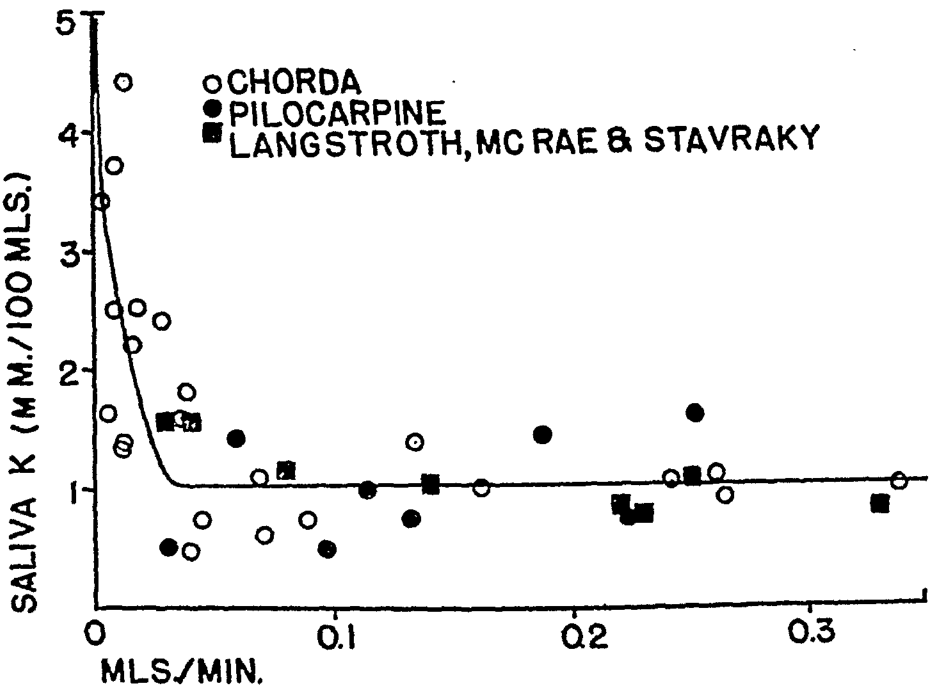

Fig. 5. Changes in the potassium concentration in saliva with changes in the rate of saliva secretion with electrical and pilocarpine stimulations of the submaxillary gland.

low rates the saliva was made up of a greater proportion of intracellular water, with a high potassium content, than at higher rates. Or it might mean that there was reabsorption of water without potassium in the ducts, as suggested by Langstroth, McRae and Stavraky (1938). It seems impossible at present to decide which of these two notions is the more correct one.

SUMMARY

The optimal frequency for electrical stimulation of the submaxillary glands of cats through the chorda tympani was about 9 per sec. Long-continued stimulation of the gland gave a series of changes in rate of secretion analogous to the changes in tension of a muscle under similar conditions.

Pilocarpine stimulation of the submaxillary gland produced a greater saliva flow for a given blood flow than did chorda stimulation. This is believed to explain in part the exhaustion of the potassium content of the gland during secretion after pilocarpine administration.

The potassium concentration of the saliva was found to increase markedly at rates of secretion below 0.03 ml. per min. This could be due either to reabsorption of water from the ducts or to a greater proportion of intracellular water in the saliva.

Acknowledgment. The thanks of the author are due to Drs. W. O. Fenn and E. F. Adolph for kindly encouragement and advice during the composition of this paper, and to Drs. W. B. Cannon and A. Rosenblueth, of the Department of Physiology of Harvard Medical School, for aid in the interpretation of the records of the experiments with long-continued stimulation of the chorda tympani.

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THE KINETICS OF LUNG VENTILATION

An Evaluation of the Viscous and Elastic Resistance to Lung Ventilation with Particular Reference to the Effects of Turbulence and the Therapeutic Use of Helium

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The amount and nature of the work done in ventilating the lungs has not been studied extensively. In general, interest has been centered upon the elasticity of the lungs or the state of constriction of the bronchi and bronchioles. In some instances intrapleural pressures have been measured but ordinarily not simultaneously with the volume changes during respiration. Wirz (1), however, has published results of a few simultaneous recordings of pressure and volume changes and has estimated the work involved in lung ventilation in the intact animal breathing air. His attention was diverted to the study of lung elasticity and Neergaard and Wirz (2, 3) described simultaneous measurements of intrapleural pressure and respired air velocity. They attempted to estimate lung elasticity by measuring intrapleural pressures at zero velocities of air movement at the ends of inspiration and expiration respectively. This difference was found later by Paine (5) to give sometimes negative and sometimes positive values for the "elastic index" of the lungs. Although this result may be due in part to inadequacy of methods used for recording pressure and velocity simultaneously by all of the above-mentioned workers it might also be real and be due to hysteresis effects in pathological lungs. Christie and McIntosh (4) also studied intrapleural pressures and total respiratory volumes but did not estimate work. They were primarily concerned with measures of elasticity of the human lung, as was Paine (5), who compared the reliability of the methods of Neergaard and Wirz with that of Christie and McIntosh and found the latter to differentiate normal and pathological lungs more satisfactorily. Paine published records of stimultaneous intrapleural pressure and respired air velocity changes in respiration in normal and pathological subjects, to which reference will be made later.

Bayliss and Robertson (6) reattacked this general problem and developed a method for measurement of the work of artificially ventilating the lungs of experimental animals. We have employed a modification of their

method in this study which was undertaken primarily to ascertain the mechanism of action of helium mixtures in altering the work of breathing.

Barach (7) in 1934 introduced the use of a mixture of 80 per cent helium and 20 per cent oxygen in the treatment of obstructed breathing. Since then a number of papers have been published which discuss helium-oxygen therapy (8). The advantages of such mixtures in cases of tracheal or laryngeal obstruction are reported to be immediate and dramatic. Prolonged use of helium mixtures is also reported to relieve bronchial asthma, although here the action is not so rapid. It might have been supposed that the greater ease of movement of helium mixtures over nitrogen mixtures would be due to a lower viscosity. However, the viscosity of helium is actually about 10 per cent greater than that of nitrogen (see table 1). Another explanation has been given by Barach (7) who says, "since *work* is in general proportional to the density, the pressure required to move

TABLE 1

Gas constants calculated for 37° from data in the handbook of chemistry and physics

	N POISES	N RELATIVE TO AIR	d	d RELATIVE TO AIR	N/d	N/d RELATIVE TO AIR	MEAN FREE PATH
			grams/cm.³				*cm.*
Air..........	189×10^{-6}	1.00	114×10^{-5}	1.000	1.66×10^{-1}	1.00	
N_2..........	187	0.99	111	0.975	1.68	1.01	8.2×10^{-6}
O_2..........	214	1.13	126	1.105	1.70	1.02	8.8
H_2..........	100	0.53	7.9	0.0693	12.65	7.61	15.4
He..........	208	1.10	15.7	0.138	13.24	7.97	33.1
80 He-20 O_2..	210	1.11	37.8	0.332	5.56	3.35	

helium-oxygen mixtures in and out of the lung, should be decidedly less than nitrogen-oxygen mixtures." This statement is not adequate as a generalization since it ignores viscance. He also pointed out (9) that passage of air into normal lungs is practically effortless, and that substitution of helium for nitrogen in gas mixtures produced no change in intratracheal or intrapleural pressures in normal breathing. He showed, however, that when there is an obstruction in any part of the air way, increased gradients become necessary to move air past the obstruction. In 1936 and 1937 (10, 11) he demonstrated a reduction in pressure gradients under certain conditions when a helium-oxygen mixture was substituted for air.

It is possible that the rate of diffusion of CO_2, which is faster in helium-oxygen than in air, might be a contributory factor in the therapeutic value of helium, since a smaller tidal volume would be required to carry off the CO_2 if its rate of diffusion in the alveolar ducts were a limiting factor. Attention has also been drawn to the fact that diffusion of a gas is inversely

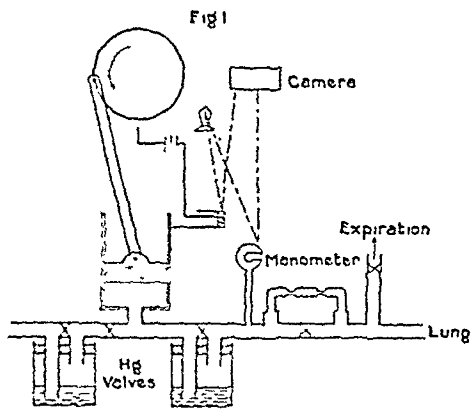
proportional to the square root of the density (7, 8). However, so far as we can ascertain, little or no attention has been paid to the conditions of gas movement in the lungs with regard to whether the movement is by Poiseuille flow, diffusion, or turbulent flow. Such information is essential before one can determine the mechanism by which helium mixtures facilitate air movement under specific circumstances.

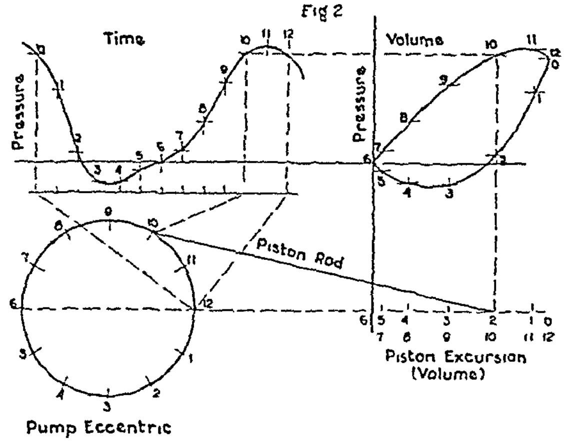
METHODS. Dogs, ranging in weight from 12 to 16 kgm., were anesthetized with sufficient nembutal to produce medium surgical anesthesia. The animal was placed on its back, the trachea cannulated and the tracheal cannula connected to a respiration pump. Where experiments were done with a closed thorax, curare (Merck) in 0.9 per cent NaCl was injected until the animal showed no spontaneous breathing movements when the pump was stopped for 30 seconds. The chest was opened by means of a mid-line incision taking care to produce as little hemorrhage as possible. The external mammary arteries were ligated and cut, and the chest walls retracted.

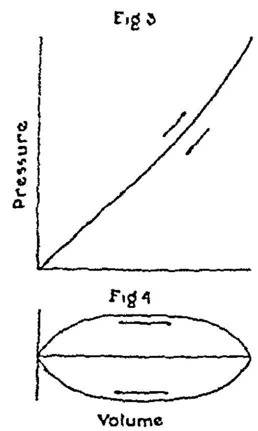
The respiration pump was one which has been used in closed circuit oxygen consumption measurements on heart lung preparations. The piston has a bore of 6.35 cm., a maximum stroke of 8.56 cm. operated by a 16.80 cm. piston rod on an adjustable eccentric. The maximum stroke was 269 cm^3. The pump was driven by a $\frac{1}{4}$ H.P. electric motor through an adjustable friction wheel and reducing gears so that the duration of the stroke could be varied between 3 and 12 seconds. The pump was arranged to close an electrical circuit actuating an electromagnet causing a spot of light to be reflected onto the camera precisely at the bottom of each stroke. (Fig. 1 shows diagrammatically the connections used.) Between measurements the pump was arranged to work through suitable valves to maintain respiration.

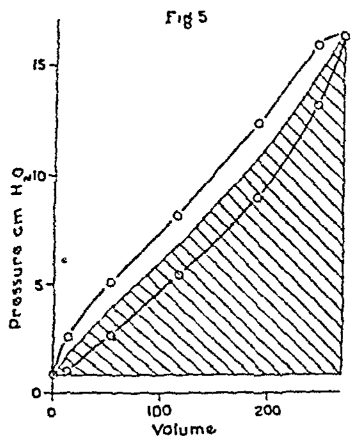
When measurements were being made, both valves were by-passed by removing hemostats. Half a stroke later the intake tube was clamped with another hemostat close to the pump and the exhaust tube was clamped. The two last mentioned hemostats were applied as nearly synchronously as possible at the top of the stroke corresponding to maximum expiration. A T-tube between the constriction shunt and the pump led to the recording manometer. When a constriction was used, it was placed in parallel with the tubing between the manometer lead and the exhaust T-tube so that all the air moved could be shunted through the constriction by clamping the main line. All rubber and glass tubing used, except for the manometer connection and the constriction, was 1.5 cm. in diameter. All joints were sealed with glycerine.

A glass spoon manometer (12) about 3 cm. in diameter was arranged to reflect the image of straight filament onto a moving paper camera. A timer was arranged to mark tenths of a second by illuminating the camera









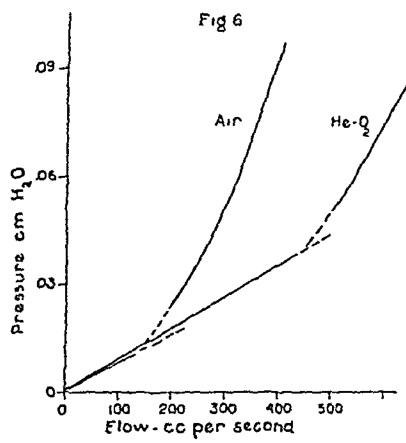


Fig. 1. Diagram of the respiration apparatus. Points where hemostats were applied are marked *X*.

Fig. 2. Diagram of the method used to construct pressure volume curves from pressure time records and the geometrical constants of the pump.

Fig. 3. Pressure volume diagram for a theoretical elastic system without viscance.

Fig. 4. Pressure volume diagram for a theoretical viscous system without elastance.

Fig. 5. Pressure volume diagram for air in a dog lung. The area of the closed *loop represents viscous work and the shaded area represents the elastic work of* inspiration.

Fig. 6. Viscous and turbulent flow. These curves were calculated from the equations:

$$P_s = \frac{128\ \eta\ L\ Q}{\Pi\ D^4} \qquad P_t = \frac{64\ k\ d\ Q^2\ L}{\Pi^2\ D^2}$$

P_s is the pressure in streamline flow, P_t is the pressure in turbulent flow, n is the viscosity, d the density, D the diameter of the tube, L its length, and Q the rate of flow of gas in centimeters^3 per sec. D was taken as 1 cm. and L as 10 cm. k was given the mean value 0.008. P is given in dynes and has been converted to centimeters of water by the factor 0.00102.

through a slotted disk, rotated by a synchronous motor. The manometers were calibrated by means of a water-filled U-tube and were linear with an error of less than 5 per cent over the useful range. A typical manometer had a natural frequency of 68 cycles per second. Manometers of this type are superior to rubber tambours since they have a linear response to pressure and can be made with an extremely small inertia.

Figure 2 shows diagrammatically the method of analyzing a record. The space between two marks, indicating full inspiration, was divided into 12 (or 24) intervals of equal duration corresponding to 30° (or 15°) rotation of the pump. The pressures corresponding to each of these times were measured making use of a graph of pressure against deflection for conversion. The volume displaced by the pump at each time interval was determined graphically as shown in figure 2 and was subsequently checked by trigonometric calculation. The relation of volume to time was not sinusoidal because of the construction of the pump. It deviated 7 per cent from a true sine wave at mid-stroke for the largest volume. The pressure was then plotted against volume to produce a loop which was closed if the pressures at two consecutive maximal inspirations were identical. Areas were obtained from the records by means of a planimeter. In case the loop was not closed, the area was corrected by the area of a triangle having as its base the difference of the final pressures, and as its altitude the total volume of the pump. This is practically equivalent to adding a linearly changing value to all points on the curve so that the ends coincide. In no case did we plot a curve which failed to close by more than 0.5 cm. H_2O and the correction never exceeded 5 per cent of the total area. Figure 8 is a photograph of four parts of a record which have been plotted as pressure volume curves in figure 7.

Results and discussion. a. *Theoretical considerations.* Bayliss and and Robertson (6) introduced the terms viscance and elastance. These quantities differ from viscosity and elasticity in that they are properties of a system and not properties of a substance. For example, the viscosity of water at a given temperature is a constant but the viscous resistance to a unit velocity of flow of water, or the viscance, depends on the dimensions of the tube through which the water is flowing. Similarly the elasticity of a gas obeys Boyle's law but the pressure produced in a gas for unit change of volume, or the elastance, is a function of the volume of the gas.

The work of ventilating the lungs is eventually all dissipated as heat produced by the viscance of the air and the tissues of the body. However, during inspiration some of the work is done against elastic forces. The potential energy stored by these elastic forces, including work done in lifting parts of the body against gravity, is partly used during expiration to move the air and tissues against their viscous resistance. With active expiration, additional work is supplied by the respiratory muscles, whereas,

if expiration is restrained, some of the energy stored will be returned to the respiratory muscles. Energy returned to a muscle is converted into heat and wasted and in addition the muscle must do extra work (13). Work can also be stored during the early phases of inspiration and expiration as kinetic energy of motion of the air and tissues; this energy is liberated during the later stages and has the effect of reducing the maximal elastic pressure in the lung. The magnitude of this inertia effect in the air is. however, very small because of the small masses involved and amounts to a maximum pressure of less than 0.5 mm. of water at the maximum frequencies and pump volumes used in this work. In any case, since the energy used to impart momentum to the gas and lung tissues at the beginning of the stroke is all regained at the end of the stroke, it is not wasted and requires no net energy expenditure by the driving mechanism.

The pleural cavity, when the chest is relaxed, is normally at a negative pressure (about 5 cm. H_2O below atmospheric). During inspiration this negative pressure increases, and at its peak may be increased by 5 to 25 cm. H_2O (14). We have assumed that it will make no appreciable difference whether inflation is produced by lowering the pleural pressure or raising the tracheal pressure; if the differences in pressure are the same, the effects would be the same so long as we can consider the air to be incompressible. Since 25 cm. of water is about 2.5 per cent of an atmosphere, the compression or expansion will be only 2.5 per cent and can probably be neglected for this work. We recognize that time is involved in movement of gas during pressure equalization and that for this reason a slight difference between positive tracheal pressure and negative intrapleural pressure inspiration is to be expected.[1]

If the trachea is connected to a simple valveless pump and pressure in the trachea is recorded, it is possible to evaluate the viscous work of ventilation as well as the work stored in elastic tissues during inspiration. To do this the pressure is plotted on a vertical axis and the volume of air displaced by the pump on a horizontal axis for one complete respiratory cycle. To understand the meaning of curves of this sort, it is useful to consider some elementary models.

If the lung be replaced by a perfectly elastic container, the pressure will be determined only by the volume of the pump and the inspiratory and expiratory curves will be identical as in figure 3. In the ideal case where Hooke's law is obeyed this curve will be a straight line. A practical model of this case is a large rigid bottle where the elasticity is furnished by the gas itself.

[1] The differences between positive and negative lung pressures become extremely significant, of course, when their effects on the blood supply of the entire thorax are considered (15).

If the lung is replaced by a non-elastic accordion or bellows connected to the pump through a constricted tube, the pressures will depend on the velocity of air in the system. This is zero at the minimum volume, increases to a maximum during inspiration, and returns to zero again at maximum volume. During expiration the velocity follows a similar course returning to zero at the end of the stroke. The pressure will vary as shown in figure 4. The area of the loop measures the integral of PdV, which is work, and is expressed in gram centimeters if the pressures are measured in grams per square centimeter and the volume in cubic centimeters (1 gram centimeter = 0.0000234 gram calories).

The lung can be compared to an elastic accordion containing some viscous resistance. Figure 5 shows a curve obtained from a normal dog lung. It can, as a first approximation, be considered as the sum of the curves 3 and 4. The area of the loop again represents the work done against viscous resistance in the lung. In order to determine how much work has been stored elastically during inspiration, we may assume that the viscance at any volume depends only on the velocity of flow and not on its direction. Then the theoretical curve of elasticity will be the median line of the loop and is shown dotted in figure 5. The work stored elastically during inspiration is then the area between this curve and the axis of zero pressure which is indicated by shading. It is also equal to the entire area above the axis in figure 5 minus half the area of the loop. Zero pressure must be taken as the pressure of minimum volume of the lung for the purposes of these calculations. Although the assumption of symmetrical distribution of viscous pressures does not hold in all cases, as will be shown, the errors are probably not very great when the work of the whole cycle is considered.

The viscous work of ventilating the lungs, which may be defined as the dissipative work of ventilation, can be considered to consist of two parts. One part is contributed by the air and represents the air viscance in the airways of the lung. The other part is contributed by the tissues and contains the viscous resistance to movement of the tissues as well as any components possibly introduced by muscular action within the lung.

b. *Modes of gas movement.* Gaseous movement can occur in three ways, by diffusion, by streamline or Poiseuille flow, and by turbulent flow (16, 17). When the orifice through which gases are flowing has a diameter comparable to or smaller than the mean free path of the gas, diffusion will be the dominant form of gas movement. The rate of diffusion of a gas is proportional to the pressure and inversely to the square root of the molecular weight. In air or nitrogen the mean free path of the molecules is of the order of 0.0001 mm., while helium at atmospheric pressure has a mean free path of 0.003 mm. (table 1). The diameter of the smallest tubes occurring in the lung is 0.1 mm. (19) so that it seems unlikely that diffusion

is directly involved in actual mass movement of air in the lung, as suggested by Barach (7). To test this hypothesis further we compared the rate of escape of a fixed volume of air and helium at the same pressures through various resistances. The gas was contained in a gas pipette over saturated NaCl. The resistance was attached to the top of the bulb and flow was produced by raising the leveling bulb producing a maximum pressure of 30 cm. of water. When a glass capillary about 0.1 mm. bore was used, the time of escape of air was about 10 per cent less than the time for helium. This is what would be expected, since air has a slightly lower stream-line viscosity than helium (see table 1). Since a straight capillary is not a very satisfactory model for the lung, we also tried a long glass tube, 5 mm. in inside diameter, packed with NaCl crystals which varied from 0.10 to 0.15 mm. on a side. This provided a number of contorted passages less than 0.1 mm. in diameter which is, according to Miller (19), smaller than the smallest bronchioles in the lung. This model showed indistinguishable rates of escape of air and helium. It is possible that there was some diffusion in this model, but since the rate of diffusion of helium is 2.7 times the mean rate of diffusion of air, there can not have been more than 5 per cent of the movement by diffusion. It can be concluded that diffusion does not contribute materially to the mass movements of air in such systems. As a further check a sintered glass filter having pores of the order of 0.03 mm. was used. In this case helium escaped 1.43 times as fast as air. There was still some indication of Poiseuille flow since this ratio is smaller than would be expected if diffusion were the only form of gas movement.

Streamline flow of gas obeys Poiseuille's law. The pressure is proportional to the rate of flow and the viscosity of the gas. The only gases with viscosities sufficiently different from air to be useful in studying streamline flow are the inflammable gases, hydrogen, methane, and ethylene which have lower viscosities than air, or the very rare gases, neon and krypton, with higher viscosities. Hydrogen is probably the best choice and was used by Bayliss and Robertson (6) in a mixture with 20 per cent oxygen. This mixture is highly explosive and we have employed it in only a few experiments discussed later.

Turbulent flow of fluids obeys a different law from either diffusion or streamline flow. The pressure necessary to produce turbulent flow varies approximately as the square of the velocity of fluid moved, and as the density of the fluid (16). There is a certain critical velocity of flow below which the flow is streamline and above which streamline flow is unstable and will become turbulent if disturbed. This critical velocity depends upon the shape of the conducting system. For a given geometrical system, however, the critical velocity of flow is proportional to the viscosity divided by the density of the fluid. Although these laws have been developed for

incompressible fluids, they hold to a high degree of accuracy for gases as well. Helium with its slightly higher viscosity and much lower density will flow faster than air before its flow becomes turbulent. At velocities of flow where air is turbulent the pressure, necessary to maintain the flow of helium-oxygen mixture, will be less than the pressure to maintain the same flow of air. Figure 6 shows the calculated pressures for 80 per cent He, 20 per cent O_2, and for air in the same system. The viscosity of the helium-oxygen mixture is taken as 1.11 and the density 0.33, both relative to air.

It will be seen that below the critical velocity and for a small distance above it, the pressure on the air is 10 per cent less than on the helium-oxygen mixture. But above this point the air requires more pressure than the helium-oxygen mixture. The ratio of pressures necessary for equal flow increases until the helium becomes turbulent. Above this velocity helium-oxygen mixtures require 0.33 of the pressure for an equal flow of air.[2]

Breath sounds which are caused by air in vibration indicate that the flow of air is turbulent, at least at the point of origin of the sounds. Therefore it is to be expected that noisy labored breathing would be relieved by the use of helium-oxygen mixtures, provided that a large part of the effort of breathing is being expended against gas movement. The critical velocity of flow depends inversely on the diameter of the conducting tubes, other things being equal. Now the bronchioles of the lungs become progressively smaller with each division, yet their total cross sectional area increases (20). Gas flow must therefore be slower in the distal bronchioles. Both reduced velocity and smaller diameter of the conducting tubes will tend to eliminate turbulence in the distal tubules so long as the latter are not partially occluded, as by mucus. Sharp flexures in the conducting tubes will, of course, produce turbulence at lower velocities, but the air ways of the lungs show very smooth junctures of a type which should discourage turbulence. Turbulence is therefore probably confined to the air passages in the head, the larynx, the trachea, and the larger bronchi. Roher (17) comes to the same conclusions from measurements on models. Figure 6 shows that the critical velocity of air flow in a 1 cm. tube is 130 cc. per second, which is similar to the conditions existing in the secondary bronchi of a dog, or the tertiary bronchi of man. The pressure drop necessary to maintain this flow in a tube 10 cm. long is 0.1 mm. of water. It would seem unlikely, therefore, that turbulence could contribute very much resistance to quiet breathing in normal lungs. Also, these considera-

[2] The above simplified discussion of turbulence is derived from Franklin and Grantham (16). Actual critical velocities depend to some extent on the smoothness of the conducting tubes, and the value of k used in figure 6 is not a true constant. The qualitative conclusions, however, are unaltered by a more exact treatment.

tions show that an obstruction in the proximal bronchi or the trachea is much more likely to produce resistance by turbulent flow alone, and, in fact, cases of just this sort are said to receive the most immediate benefit from the use of helium-oxygen mixtures. Schultz and Jordan (21) showed that, in the guinea pig, anaphylactic shock caused constriction in the secondary bronchi. Contractions which occur at this level would be much more likely to cause turbulence and abnormal breath sounds than contractions of the terminal bronchioles.

c. *Turbulence and the effects of helium.* It is possible to determine whether turbulence plays any significant rôle in the work of ventilating the lung by substituting a mixture of 80 per cent helium—20 per cent oxygen for air. We have done this experiment 21 times on 4 dogs with normal airways and have never found any significant difference of viscous work between the two mixtures. It is usually impossible to detect any difference whatever in the curves, provided that the filling of the pump with air and with helium is done at the same pressures.

However, when a constricted tube, designed to produce turbulence as shown in figure 1, is placed in series with the trachea, the pressures accompanying ventilation of the lung are altered and the viscous work is increased. When a helium-mixture is substituted for air this work is markedly decreased. This fact has been verified in numerous trials. Figure 7 shows the results of a typical experiment upon the effects of air and helium through the same constriction, as well as a record without the constriction which shows identical work for air and helium. The original pressure tracings from which these curves were drawn are shown in figure 8. The effect of substituting helium for nitrogen is apparent in the pressure tracings as well as in the pressure volume diagrams. However, the area of the loop of the pressure-volume diagram is a direct measure of the work done during the cycle and is therefore directly correlated with the effort of breathing. It is possible to have large changes in the area of the pressure-volume diagram with only negligible changes in the maximal and minimal pressures.

These observations prove that air viscance in obstructed larger airways is diminished by substituting helium for nitrogen. Since helium has a higher streamline resistance but a lower turbulent resistance than nitrogen it is obvious that the lower work in moving the helium mixture is due to its higher critical velocity and lower turbulent viscosity.

This effect is very large when constrictions are placed in the upper airways. In order to test the effect of airway constriction at other points upon turbulence, pilocarpine, 1 mgm. per cent in physiological saline, was administered intravenously to a dog after control studies of ventilation work had been made under standard conditions. An initial dose of 10 mgm. caused profound cardiac slowing and injection was continued with a continuous injector at the rate of 0.5 mgm. per minute. Pressure-

volume curves taken before and after injection showed a 60 per cent increase in elastic work. Substitution of helium-oxygen for air after pilo-

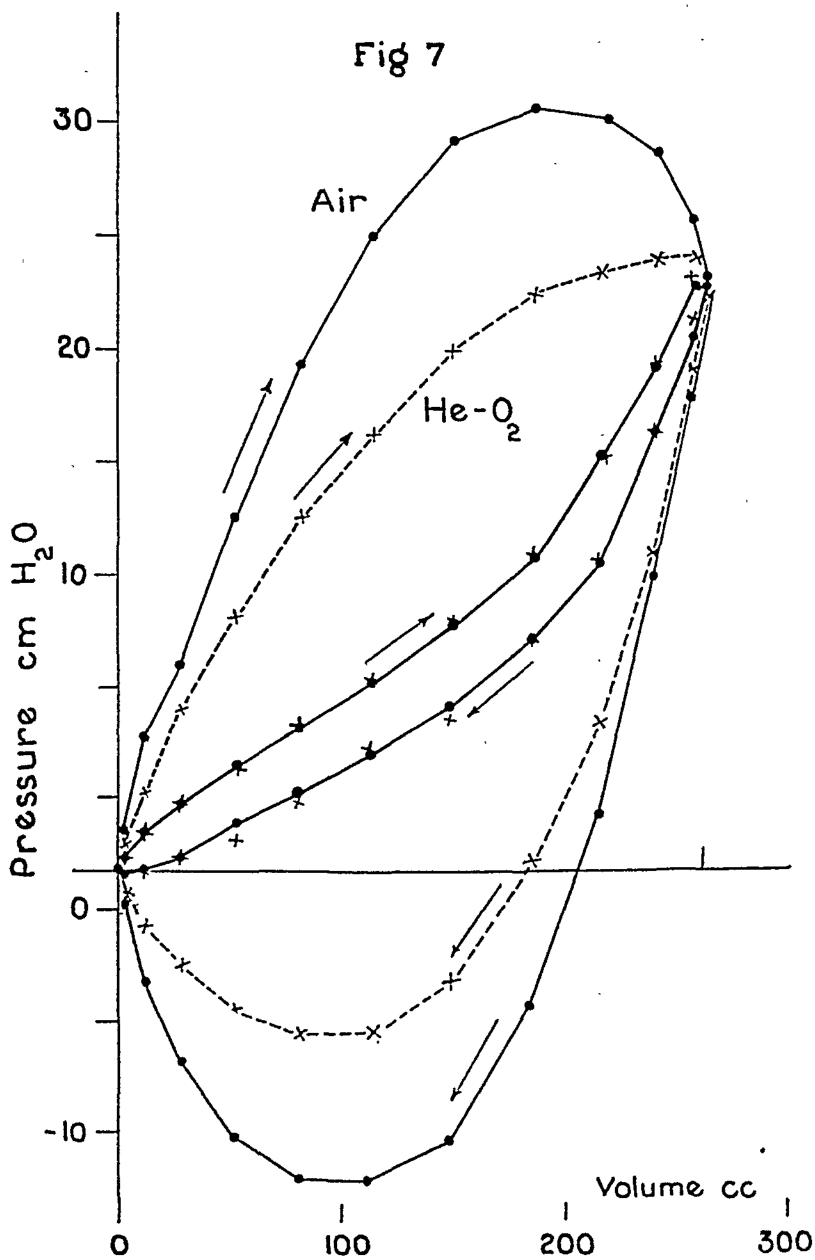
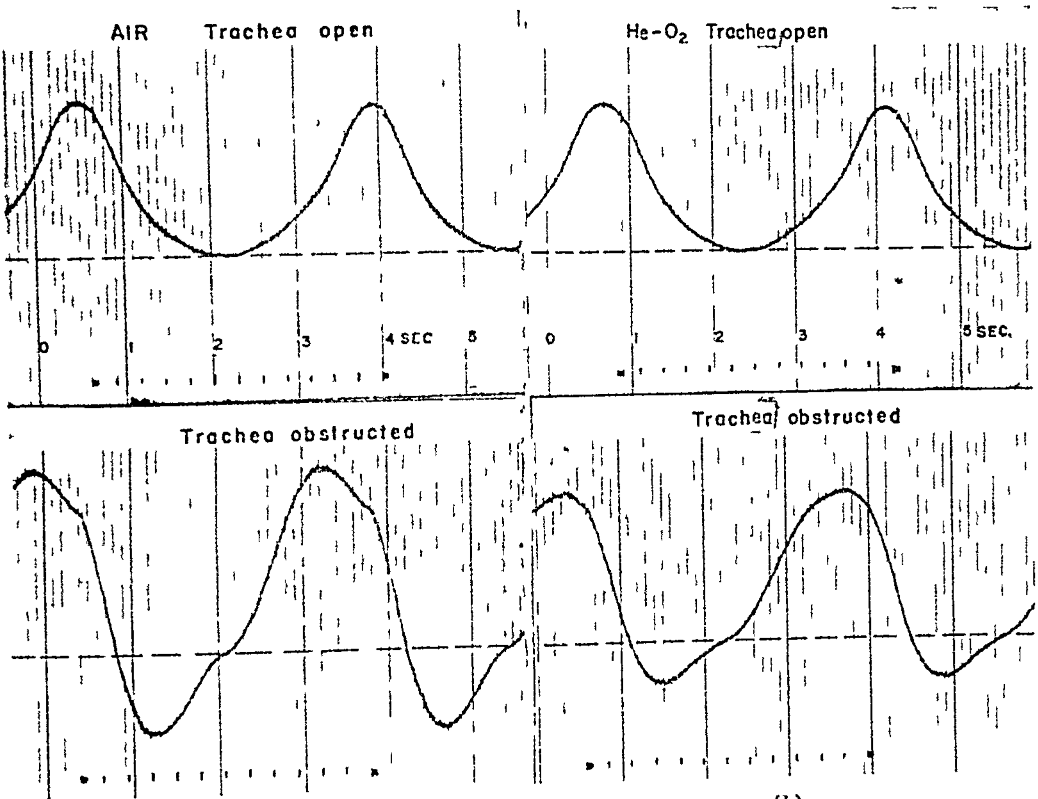


Fig. 7. Pressure volume diagram with and without tracheal obstruction. The central loop marked with • and + represents air and helium oxygen with no obstruction. The outer loop marked with • represents air with a constriction as shown in figure 1. The middle loop marked with + represents helium-oxygen through the same constriction.

carpine left the elastic work unchanged but decreased the viscous work 10 per cent (table 2). These results confirm the observations of Bayliss and Robertson (6), who found small alteration in gas viscance by sub-

stituting hydrogen for nitrogen after pilocarpine administration. Both sets of observations indicate that increase in gas viscance is a small factor



(a) (b)

Fig 8. Time pressure records from which figure 7 was determined. The left margin of the solid black marks on the lower part of each record represents the instant the pump reached the bottom of its stroke corresponding to maximum inspiration.

TABLE 2

Work of ventilation before and after pilocarpine—Tidal volume 261 cc., respiration rate 20 per min

	VISCOUS WORK V	TOTAL WORK T	ELASTIC WORK $E = T - V/2$
	gm cm	*gm cm*	*gm cm*
Before drug: Air or He-O_2	1980	3152	2162
After drug:			
Air	1704	3980	3028
He-O_2	1540	3820	3050

in the increased resistance to breathing following pilocarpine, and that tissue elastance and viscance are more important factors.

Since without obstruction to airways we have found turbulent gas vis-

cance to be below the limit of detection we can conclude that gas viscance in normal lungs follows Poiseuille's law and should vary linearly with velocity. Tissue viscance is a more complex function, but it is in part composed of simple fluid viscance. Other factors undoubtedly enter in and it seemed to us to be possible that the importance of those other factors which are connected with structural plasticity might be evaluated by measuring the work of ventilating the lungs at various speeds.

A number of experiments were therefore done at slow and fast pump speeds. Three of these which seem to be typical and which were uncomplicated by breathing movements were measured and the results are listed in table 3. While the results show rather wide variations in different animals and under various conditions, it is evident that the reduction in

TABLE 3

Work of ventilation in relation to velocity—Tidal volume 269 cc.

EXPERIMENT	VISCOUS WORK, V	TOTAL WORK, T	ELASTIC WORK, $T - V/2$	DURATION OF STROKE, S	FREQUENCY, $f = 1/S$	$V_1 - V_0 = f_1 \frac{V_1 - V_2}{f_1 - f_2}$	V_0	$\frac{V_0}{V_1} \times 100$
	gm. cm.	gm. cm.	gm. cm.	seconds		gm. cm.	gm. cm.	per cent
A_1	918	1920	1461	2.87	0.348			
A_2	608	1840	1536	9.9	0.101			
$A_1 - A_2$	310				0.247	437	481	52
B_1	600	2155	1855	2.85	0.351			
B_2	534	2110	1843	10.75	0.093			
$B_1 - B_2$	66				0.258	90	510	85
C_1	790	1855	1490	2.90	0.345			
C_2	560	1684	1424	4.80	0.208			
$C_1 - C_2$	230				0.137	575	215	28

total viscous work is not proportional to the frequency of the pump. There must be some fraction of the viscous work of ventilation which is relatively independent of velocity. This work can not be in the gas nor in the viscance of ideal fluid in the lung tissue for the reasons outlined above. It may be deduced that tissue resistance related to structural plasticity accounts for a large fraction of the viscous work. We have calculated the magnitude of this component of tissue resistance on the assumption that the Poiseuille viscous resistance of gases and liquids in the lung is linearly proportional to frequency and is added to the plastic resistance. The results as shown in columns 8 and 9 of table 3 indicate clearly that a considerable fraction of the viscance does not vary linearly with velocity. There is, of course, no *a priori* reason to suppose that the plastic resistance will be a constant quantity or a constant fraction of the

total viscous resistance. Bayliss and Robertson (6) found essentially the same thing; a component of viscance which was largely independent of frequency. They also report that the total tissue viscance at 18 cycles per minute accounted for about two-thirds of the total viscance. Our results confirm theirs to within the accuracy of the determinations.

d. *Hysteresis effects and elasticity.* We investigated the effect on the pressure-volume diagram of altering the residual volume of the lung by connecting a 100 cc. syringe to the line so that air or gas mixtures could be added or removed. Figure 9 shows the effect on the diagram when two successive additions of 100 cc. were made. The diagrams shown here were taken 3 to 6 seconds after injecting the air into the system, and each cycle required 2.8 seconds. The abscissal values for the second and third loops were taken as the respective pump volumes plus the amount injected. If the lungs behaved as a simple elastic system, the median lines of the three loops would all lie on the same line. Actually, the pressure produced by adding 100 cc. 6 seconds earlier is not as great as the average pressure when the pump has put 100 cc. into the lungs which required only 0.65 second at the speed employed. The following experiment was performed to elucidate the phenomenon further. The pump was stopped and 100 cc. of air was injected every 5 seconds, the injection taking 0.6 second, until 500 cc. had been injected, then 100 cc. was removed every 5 seconds. Pressure was continuously recorded, and it was found that there was a rise of pressure during the injection, followed by a gradual fall, until the next injection was made. Figure 10 shows the result of one of these experiments, done on a living dog with the chest open. Practically all the drop in pressure at constant volume took place in one second. It will be seen that there is a hysteresis effect in that the pressure on deflation is less than the corresponding pressure on inflation. This hysteresis effect has the same influence on the pressure-volume diagram as does viscance when the pump is moving continuously. However, in this experiment there was no gas movement during the equilibration process. A number of step inflation experiments were done on the lung of a dog *in situ* soon after death. No precautions were taken to keep the temperature above that of the room. The dying lung shows a much greater hysteresis effect than the living lung (fig. 11), and part of the decay of pressure continues after the first second.

This effect could possibly be qualitatively explained by assuming that the elasticity of the lungs is in two sets of chambers, separated by a high viscance. Then if air is rapidly introduced into the first set of chambers, this would cause a high pressure which would fall as the air flows into the second set. However, when we consider the size of the available chambers and tubes, the time required to reach equilibrium seems much too long. It is possible to rule out this hypothesis by substituting pure hydrogen for the air in the lung. Hydrogen with its lower viscosity and

much higher critical velocity should decrease the time necessary for equilibrium. Figure 12 shows the effect of step inflation of the same lung with hydrogen only 400 cc. was injected, and the resting volume must have been somewhat larger since the curve does not exactly coincide with figure 11; but the difference is not significant. The fall in pressure immediately following the injection of hydrogen is in fact somewhat larger than for air.

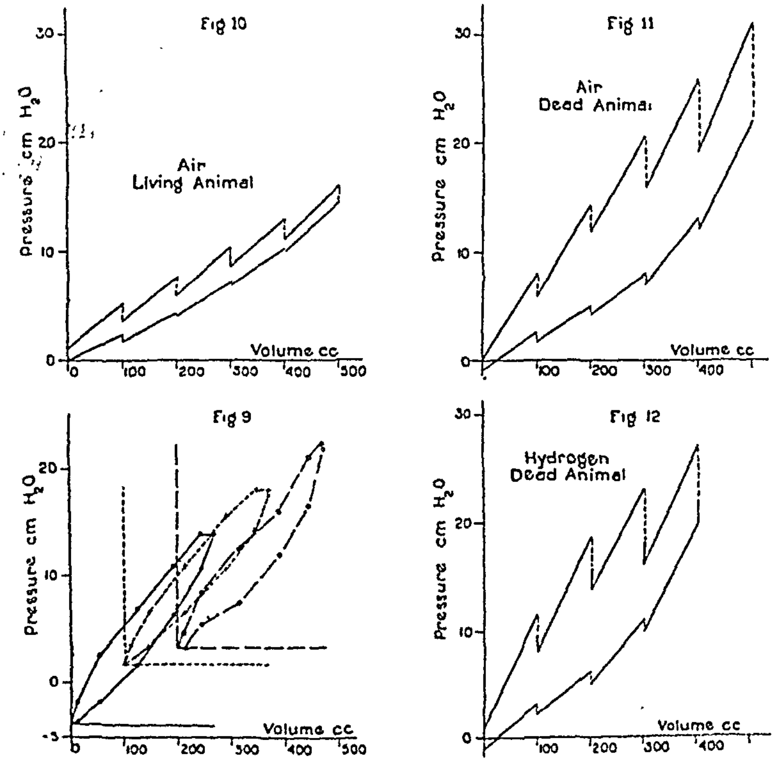


Fig. 9. Pressure volume diagram before and following two separate additions of 100 cc. of air.

Fig. 10. Pressure volume diagram for air in a live lung with the chest open. The change in pressure at constant volume occurred during about 1 second after each change in volume.

Fig. 11. Pressure volume diagram for air in a dead lung. The change in pressure during the first second is shown as a dotted line, during the remaining 3.5 seconds as a solid line. Changing the volume took 0.5 second and was done every 5 seconds.

Fig. 12. Same as 11 using hydrogen.

The effect is not due to asphyxia of the lung by hydrogen since pure nitrogen was practically indistinguishable from air in its effect. We suggest that the higher initial reaction of the lung to hydrogen is a result of the fact that the hydrogen could be injected more quickly than air because of its lower viscous and especially turbulent resistance.

The response of the lungs to a change in pressure of the type described above is a hysteresis effect. The hysteresis or accommodation effect manifests itself as a gradual change in residual air volume following a

maximal inspiration. Christie and McIntosh (4) use such a change as an indication of "set" or plasticity which they claim occurs only in pathological and not in normal lungs, although it is a fairly common observation following exercise on a metabolism apparatus. In any case their methods would not detect small hysteresis effects such as we have measured. The much larger hysteresis effects which we found in the lungs deprived of circulation might be considered as a form of pathological response. Hysteresis has been noted in the dead lung by Hirakawa (24).

As would be expected, opening the chest reduces the elastic work of respiration. Table 4 and figure 13 show the changes in the several work fractions produced by opening the chest. The change in viscous work is variable but there is a consistent reduction in elastic work. The variation

TABLE 4

Work of ventilation with closed and open chest—Tidal volume 269 cc.

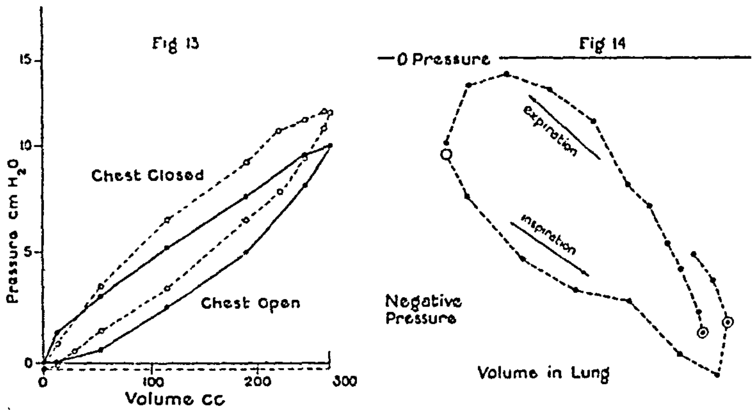
	CLOSED CHEST			OPEN CHEST			$\frac{V_1}{V_2}$	$\frac{E_1}{E_2}$
	Viscous work, V_1	Total work, T_1	Elastic work, $T_1 - V_1/2$	Viscous work, V_2	Total work, T_2	Elastic work, $T_2 - V_2/2$		
	gm. cm.	*gm. cm.*	*gm. cm.*	*gm. cm.*	*gm. cm.*	*gm. cm.*		
A	600	2155	1855	760	1885	1505	0.76	1.23
B	1000	2860	2360	520	1780	1520	1.92	1.60
C	780	2850	2460	700	2270	1920	1.11	1.28
D	620	1940	1630	720	1560	1200	0.86	1.36
E	1172	2980	2394	568	1316	1032	2.06	2.32
Mean							1.34	1.56

in work of ventilation is due mainly to the use of constant ventilation volume with dogs of differing size.

f. *Elasticity and normal breathing.* Pressure volume curves for normal breathing in the human may be calculated from the data of Neergaard and Wirz (2, 3) and Paine (5). To do this the curve of air velocity is integrated by adding the ordinates at equal time intervals to obtain relative volumes. Paine gives absolute volume changes so that the relative volumes can be calibrated. We have integrated all the curves in the papers referred to above and reproduce three of them here, figures 14, 15, 16. In this case intrapleural pressures are plotted in such a way that the curves have the opposite inclination from our other curves. They would have the same inclination if we had plotted the difference between oral pressure and pleural pressure as a positive value.

Figure 14 from Neergaard and Wirz is the only one of these curves which is similar in shape to our curves with pump respiration. Even so the

viscous work is a large fraction of the total work. Unfortunately there are no data in the original paper which will permit the calculation of either pressure or volume in absolute units. All of Paine's diagrams are more



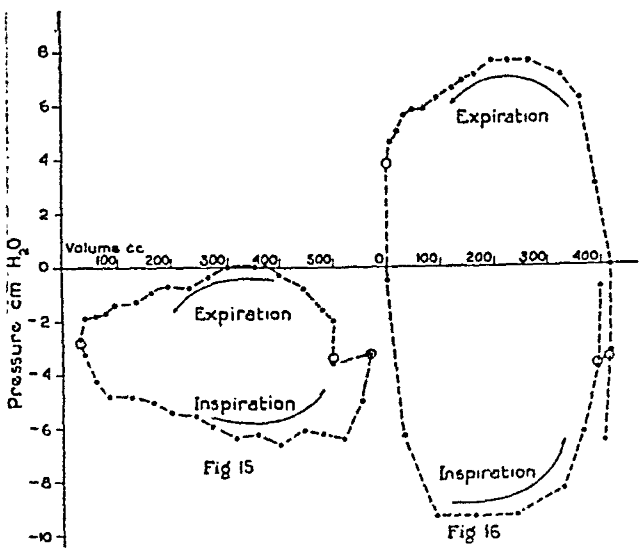


Fig. 13. Pressure volume diagrams with the chest closed and open.

Fig. 14. Pressure volume diagram obtained from the curve of Neergaard and Wirz (3) p. 61. Volume in the lung increases in arbitrary units to the right. Open circles indicate points at which the air velocity was zero.

Fig. 15. Pressure volume diagram for a patient with normal lungs obtained from the data of Paine (5), figure 4. Open circles indicate points at which the air velocity was zero.

Fig. 16. Pressure volume diagram for a patient with pulmonary emphysema. Obtained from the data of Paine (5), figure 8. Open circles indicate the points at which air velocity was zero.

nearly rectangular and the elastic component is difficult or impossible to measure with accuracy. His patients all seem to exert an approximately constant intrapleural pressure throughout inspiration and expiration. It

follows that the air velocity falls progressively as the elastic pressure in the lung approaches the intrapleural pressure since it is this difference in pressure that produces air flow. Figure 16 is a record of a patient with extensive emphysema and shows the high positive pressures necessary to produce expiration. The points are spaced at equal intervals of time and show the prolonged expiration time. Although in this case the apparent elasticity is high it is anomalous in that it indicates an intrapleural pressure greater than atmospheric at the beginning of inspiration when the air is moving into the lungs. It is most probable that the emphysematous lung has a very large plastic viscance and little if any elasticity so that the patient must use special effort to produce expiration and can not rely on the elasticity of his lungs. If this is true then helium-oxygen mixtures could produce no reduction in the work of moving equal volumes of gas in emphysema. The larger mean free path of the molecules in helium mixtures might still produce favorable effects.

CONCLUSIONS

1. The time relations of pressure and volume changes produced during the ventilation of dog lungs by a valveless pump have been measured.
2. Diagrams of these pressures against the volume displaced by the pump permit the separate evaluation of viscous and elastic resistance to ventilation.
3. The viscous resistance of the normal lung is not changed when a helium-oxygen mixture is substituted for air.
4. The viscous resistance produced by an obstruction which produces turbulence is reduced by the substitution of helium-oxygen for air.
5. It is concluded that the mode of action of helium in reducing the work of ventilation is by a reduction of turbulence in the gas flow.
6. The lung shows adaptation to changes in volume which accounts for a large part of the viscous pressure of ventilation.
7. Pressure volume diagrams calculated from data in the literature are shown for human subjects breathing naturally.

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THE INFLUENCE OF GLYCINE ON MUSCULAR STRENGTH

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Down the centuries one mark of man's prowess has been great muscular strength, and any means of increasing it has been as much sought for as the philosopher's stone. One of the most recent expedients is the addition of glycine to the diet (Chaikelis, 1941). If this does increase muscle strength, the increase would appear to be implicit in the reported rôle of gelatin, which is one-quarter glycine, in increasing work capacity on the bicycle ergometer (Ray et al., 1939). Possibly glycine, whether ingested as such or as a constituent amino acid in gelatin, has a creatinogenic action. If so, its reputed action in exercise might find an explanation, as well as its effect on muscular dystrophies (Boothby, 1934).

Most recent studies of the addition of gelatin to the diet have failed to reveal any fatigue-allaying action or any effects on work performance (Hellebrandt et al., 1940; Robinson and Harmon, 1941; Karpovich and Pestrecov, 1941; and unpublished data from this laboratory). However, the positive effects of glycine feeding on muscle strength reported by Chaikelis are so clear-cut that we decided to repeat one phase of his study.

Procedure. Eight men on our laboratory staff squeezed a hand dynamometer as a test of strength of grip morning and evening for a period of eleven weeks. After the first week, when all were on their usual diet, six subjects began receiving six grams of glycine per day while the other two received placebos. No subject knew what he was receiving with the exception of S. M. H. During the tenth and eleventh weeks, one of the latter two (R. C. D.) was placed on 12 grams of glycine daily. At the beginning of the sixth week, glycine was withdrawn from two subjects who were then placed on placebos for the remainder of the experiment. Each week a 24-hour urine was collected by the subjects. The urines were analyzed for creatine, creatinine and nitrogen by methods previously described (Dill and Horvath, 1941).

Results. The results of the strength measurements are the weekly means expressed as per cent of the control week. The data for the separate hands are shown for each subject (fig. 1).

Control subject R. C. D. had increased in strength measurement 8 to 10 per cent over the initial value by the ninth week. The ingestion of 12

grams of glycine daily during the following two weeks did not augment his performance. Control subject C. A. K. attained his highest level of

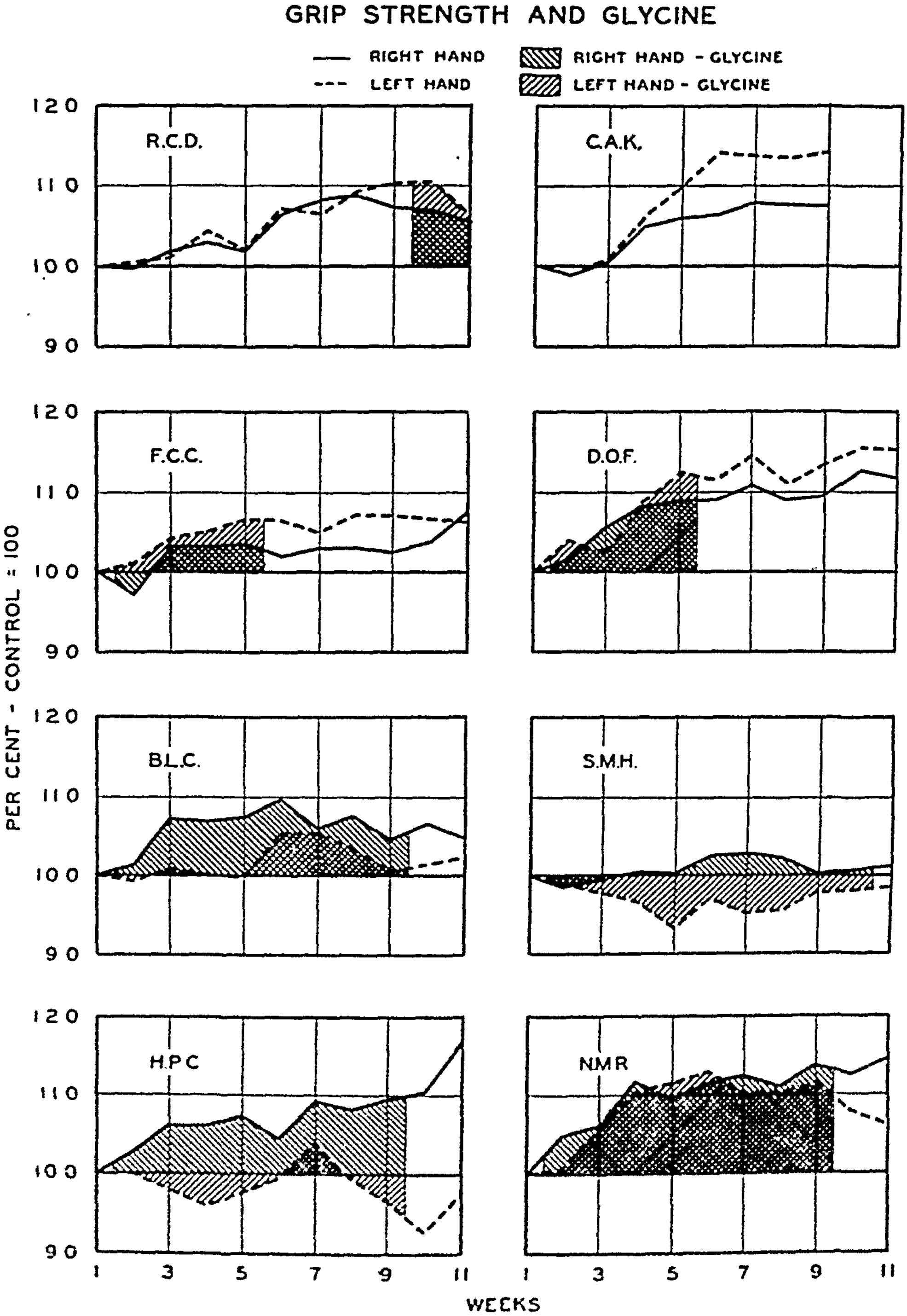

Fig. 1

improvement in six weeks and maintained it thereafter. This was roughly 8 per cent for the right hand and 14 per cent for the left.

F. C. C. and D. O. F., who received glycine for four weeks, did not improve more than control subjects. After being placed on placebos when they were withdrawn, D. O. F. exhibited during the six weeks following an actual increase, while F. C. C. remained at about the same level.

The other experimental subjects did not show greater increases in grip strength than control subjects. B. L. C.'s maximum gains were 10 per cent for the right and 6 per cent for the left hand. The grip strength of S. M. H. increased to a maximum of only 3 per cent with the right hand and showed a decrease for the left; it never reached the control level throughout the glycine period. H. P. C. had a greater right hand grip,

TABLE 1

The mean 24-hour excretion of creatine and creatinine in grams in the urine of control subjects and subjects ingesting 6 grams of glycine per day

	WEEKS									
	1	2	3	4	5	6	7	8	9	10
	Control	Placebos								
Control subjects (2)										
Creatine	0.13	0.03	0.05	0.14	0.25	0.26	0.41	0.12	0.06	—
Creatinine	2.03	1.97	1.99	2.12	2.09	1.96	2.19	2.01	1.98	—
	Control	Glycine				Placebos				After
Experimental subjects (2)										
Creatine	0.21	0.06	0.12	0.41	0.41	0.22	0.23	0.37	0.24	0.19
Creatinine	1.92	2.19	2.21	1.70	2.09	1.84	1.95	2.01	2.44	2.10
	Control	Glycine								After
Experimental subjects (4)										
Creatine	0.08	0.06	0.03	0.16	0.08	0.22	0.14	0.19	0.12	0.15
Creatinine	1.73	1.85	1.83	1.74	1.88	1.81	1.88	1.85	2.03	1.86

but a variable and usually sub-control left. He showed a spurt the second week after glycine was withdrawn. N. M. R. showed an improvement of roughly 11 and 14 per cent for separate hands.

Glycine does not increase muscle strength nor modify the course of the training curve. The changes in performance can be ascribed to training and to increased skill in using the instrument. No subject reached the mean order of strength increment recorded by Chaikelis (22–23 per cent), even though our experiment had a training factor which strengthened the grip. Chaikelis reports no intermediate tests between the initial and final ones, and records no activity which would have tended to train his subjects for this task. Ray's statement that the work performance of his subjects

declined after being deprived of an adjuvant containing glycine was not confirmed. There was no drop in the performance of the present subjects during periods of 2 to 6 weeks following cessation of glycine.

The urinary excretion of creatine and creatinine in one period of 24 hours was determined once each week, as shown in table 1. Creatinine excretion varied from week to week with possibly a slight upward trend. This was not in agreement with Chaikelis' results, where creatinine concentrations decreased roughly 30 per cent. Chaikelis' results on creatinine were obtained on urines drawn some time within six hours of a bout of exercise; the volumes were not measured. Such values indicate concentrations, not amounts. Our results, on the other hand, are in line with our previous finding that gelatin does not alter the creatinine excretion. The variation from day to day in a single subject was generally greater than the mean differences observed. In agreement with previous evidence (Dill and Horvath, 1941; Horvath and Corwin, 1941), creatine was excreted. The alterations in its excretion during the period of the study cannot be ascribed to glycine *per se*, since they were equally great when glycine was not being administered. There was a slight increase in nitrogen excretion during the period of glycine ingestion.

SUMMARY

Eight subjects were tested for strength of grip twice daily for eleven weeks. Four subjects received six grams of glycine daily for eight weeks. Two received it for four weeks and then placebos. Two others served as controls for nine weeks. One of these was then given twelve grams of glycine daily for two weeks.

The improvement in grip strength in subjects receiving glycine was no greater than that of those who were given placebos. Neither creatinine nor creatine excretion showed changes attributable to the ingestion of glycine.

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ANALYSIS OF THE INITIATION OF FIBRILLATION BY ELECTROGRAPHIC STUDIES[1]

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It has been pointed out by Wiggers (6) that ventricular fibrillation, evoked by application of a strong brief shock during the vulnerable period of late systole or of early diastole, starts with a series of 3 to 6 undulatory contractions which have many earmarks of premature beats. They are attended, in standard electrocardiogram leads, by large bizarre complexes which recur at progressively decreasing intervals and change their form in successive beats. Such complexes apparently contain a clue as to the mechanisms responsible for the induction of fibrillation. Unfortunately, however, they represent the resultant of so many ultimate electrical potentials that their further study has not seemed promising.

In this investigation, an intimate study of electrograms recorded from various small spots on the ventricular surface was primarily undertaken with the hope of elucidating *a*, the mechanisms by which strong, brief and *localized* shocks can cause a *general* disorganization of ventricular excitation necessary for the fibrillating state, and *b*, the reasons why this is only brought about, in normal hearts, by stimuli which fall during the vulnerable phase. It was soon found that this also required a restudy of single responses of the ventricles by stimuli applied at various moments of systole and diastole.

Procedures. Dogs from 8 to 15 kilos in weight and of both sexes were used as experimental animals. Anesthesia was induced by intravenous administration of sodium barbital, 200 mgm. per kilo, usually preceded by a subcutaneous injection of 1 or 2 cc. of 2 per cent morphine sulfate solution. Mild artificial respiration was instituted and the chest opened by a midline incision through the sternum. The heart was suspended in a pericardial cradle and kept moist with drip Ringer's fluid. Experiments were usually completed within four hours. The results reported are based on large numbers of observations on each of the 20 dogs employed.

[1] This work was made possible by a grant from the John and Mary R. Markle Foundation.

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Electrograms were picked up simultaneously by three pairs of contiguous electrodes recently described by Harris (2), and were recorded by three large Hindle galvanometers, properly aligned to obviate parallax. Time lines, cutting all curves, were recorded by spokes of a wheel actuated by a synchronous motor. The three pairs of contiguous electrodes were variously aligned and spaced in relation to each other and to the bipolar stimulating electrodes in different tests on the same heart, so that numerous points were studied. In some tests, the leading electrodes were arranged around the stimulating electrode as arcs of circles; at other times, linearly. An idea of the plans of arrangement in different tests is shown schematically in figure 1. One of these leads was usually placed as close to the stimulating electrodes as feasible without risk of damage to the galvanometer string. One of the advantages in using the contiguous electrodes for leads was that, by proper rotation, shocks as great as 40 M.A. could be

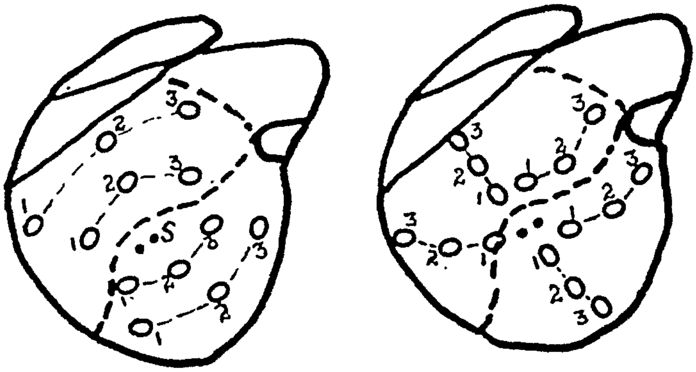

Fig. 1. Two diagrams indicating radial and linear placement of three pairs (*1, 2, 3*) of modified differential electrodes (contiguous electrodes) with respect to stimulating electrodes (S) in different experiments.

applied at a distance of 6 to 7 mm. The stimulating electrodes were also shifted in tests, the usual points selected being the apex, the left or right side of the ventricular septum, the base and pulmonary conus of the right ventricle.

In most of the observations the sinus node was clamped in order to slow the heart rate, and the heart was then driven by induction shocks applied to the right auricle. Every sixth beat, a brief D.C. shock 0.01–0.03 second in duration and ranging in strength from 5 to 40 M.A. was applied progressively earlier in a cycle through carefully plated Ag·AgCl electrodes, thus exploring the reactions at various moments of the whole cycle, systematically. For this purpose, the stimulator recently described by Wegria, Moe and Wiggers (5), which also alternated the direction of the current, was employed. It should be emphasized that shocks which evoke multiple systoles or fibrillation are several hundred-fold stronger than diastolic threshold shocks. Many special procedures, best described when pertinent topics are discussed, were also used.

Premature beats induced by late systolic stimuli. *Strong shocks* applied in late systole—determined either as mechanical systole from ventricular pressure curves, or as electrical systole of standard electrograms—evoke responses early in diastole (Wiggers and Wégria, 7). Such observations require explanation, first, because they seem to contradict the generally accepted doctrine that cardiac fractions are refractory up to the end of contraction, and secondly, because shocks which are strong enough evoke not one, but several beats early in diastole which may lead to fibrillation. In order to reconcile such findings with current knowledge, it has been suggested that this implies excitation of such myocardial fractions as have been repolarized or have stopped contracting somewhat earlier than is indicated by pressure curves or standard electrocardiograms (King, 3; Wiggers, 6). However, this meets with certain difficulties in interpretation recently discovered by Wégria, Moe and Wiggers (5). Therefore, experiments were carried out to determine whether smaller portions of ventricular myocardium respond when a shock is applied during electrical systole of localized fractions, i.e., during the Q-T segment of punctate electrograms recorded from spots adjacent to and more distant from the stimulating electrodes. As Harris (2) has recently emphasized, such electrograms represent the localized responses of a very small area of tissue under an electrode. When properly oriented in relation to the direction of normal excitation it consists of a sharp spike, the beginning of which is conveniently designated Q, and a final smaller deflection designated as T, much as in standard electrocardiograms. This Q-T interval unquestionably represents the duration of the electrical systole in very localized regions. When the direction of the impulse or rate of conduction change, the character of the deflection is also altered.

The character of these experiments is illustrated in figure 2, A, B, C, in which leads 1, 2 and 3 were taken from points similarly marked on the accompanying diagram. Brief D.C. shocks (30 M.A.) were applied as shown by the shock artefacts indicated by arrows. The three curves are taken from a long record of one experiment in which shocks were consecutively advanced from diastole to systole. They illustrate a single premature contraction after shocks delivered late in diastole (curve A), on the T wave (B), and definitely earlier than the T wave (C). The deflections are aberrant in form in leads 1 and 2, which were nearer the stimulating source, but retain their "spike characteristic" in lead 3, taken at a greater distance. The conclusion is warranted that points 1 and 2 received their excitation over abnormal, and point 1 ultimately over normal pathways.

Innumerable instances of systolic responses, such as are indicated in curve C, strongly suggest that nonrefractoriness during late systole cannot be explained on the basis of an early termination of contraction in certain areas of the myocardium. They are obviously not due to spread of current

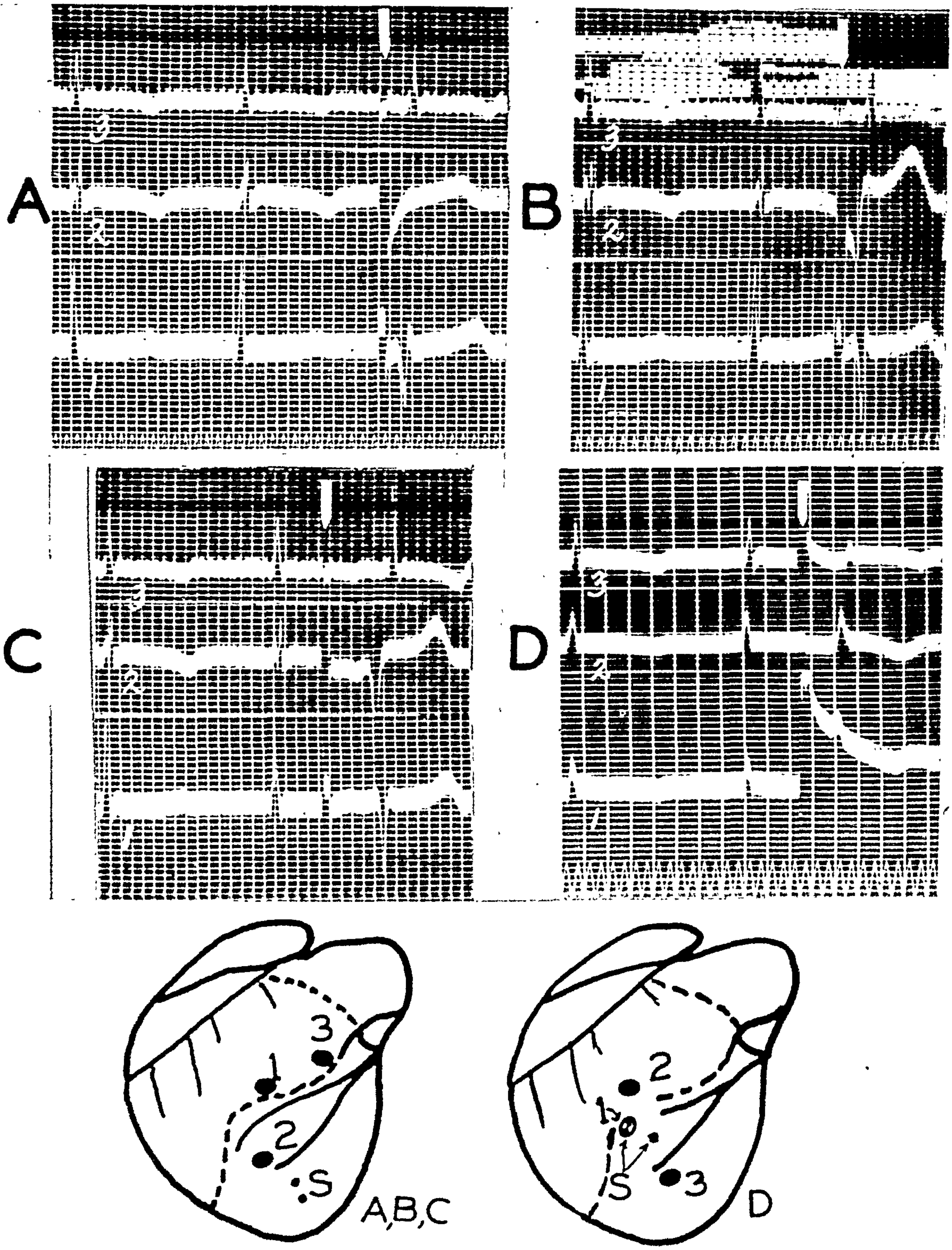

Fig. 2. Segments of records illustrating responses to D.C. shocks applied late in diastole (A), on the T wave (B), and preceding the T wave (C). Segment D shows a response in a lead taken from the stimulating electrode. Diagrams indicate location of punctate leads *1*, *2*, *3* and points of stimulation (S) in curves A, B, C and D respectively. Time, 0.02 sec. in A, B, C; 0.01 sec. in D.

to the auricle and subsequent re-excitation, as was again suggested by Woodbury (8) in the case of the turtle ventricle, for analysis of many curves invariably showed 1, that the order of initial response is related directly to the distance of leads from the stimulated points, and 2, that the contour of deflections changes in areas more adjacent to the source of stimulation, but generally remains the same in areas more remote from it.

The latency and interpunctal conduction times of single premature systoles. The latency of electrical responses occurring at different points on the ventricular surfaces after brief D.C. shocks applied at consecutive moments of the cardiac cycle as well as the interpunctate differences between points

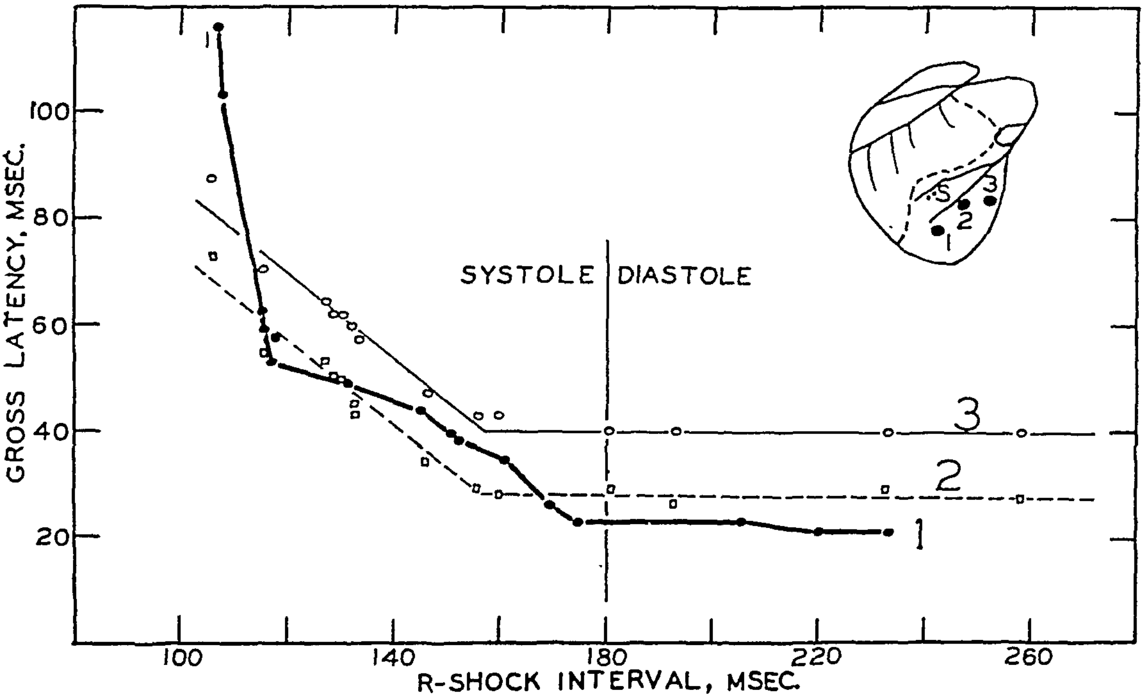

Fig. 3. Plot showing the constant latency of responses of three points (*1*, *2*, *3*) to shocks delivered during diastole, and the linear increase in gross latency as shocks are advanced into systole. Difference between lines gives interpunctal intervals. Abscissae, interval between preceding R deflection and shock.

in line with, and on the same side of, the stimulating electrodes have been measured.

Results showed that the latency of deflections resulting from shocks given during any portion of diastole and, in fact, as early as the summit of a T wave is always constant in any given lead. This does not agree with observations of Blair, Wedd and Young (1) on turtle strips in which conduction seemed to be affected by the diastolic interval. This gross latent period averages 12 to 15 msec. at points about 8 mm. from the stimulated locus and about 50 msec. at the most remote surface points on the anterior ventricular surface. Determination of latency at points near

the stimulating electrode is complicated by the shock artefact, except when low-voltage stimulation is used, in which case it can be detected at the top of the rectangular shock artefact. The interpunctal intervals likewise remain constant, regardless of the moment of diastole in which a stimulus is applied. This is illustrated by the plot of an experiment in figure 3.

When shocks are advanced so that they fall previous to the summit of T the situation changes. As indicated by the trend of lines in figure 3, the gross latency increases in linear fashion in most of the surface points as shocks are advanced in systole and may reach a magnitude as great as 100 msec. in very early shocks. In such instances, the pattern of the electrical deflections at any spot remains the same regardless of the moment at which the shock was applied. The only points which, in a few experiments, did not show such a methodical linear increase in latency were those on the apex; but in these the pattern of electrical deflections changed, suggesting involving of different conduction pathways.

Such progressive linear increase in the intervals of response might represent an apparent latency, really due to delayed conduction, or it may be a real latency of some sort. In favor of the former are many fundamental demonstrations indicating that any real latency of diastolic responses must be extremely short and perhaps nonexistent, differences being solely due to variations in conduction rates or conduction paths.

The following facts indicate, however, that delayed conduction does not suffice to explain why the latency increases linearly as strong systolic shocks are applied earlier and earlier in systole:

1. The interpunctal intervals, i.e., the time differences between points in line with and on the same side of the stimulated locus remain constant for responses to systolic and diastolic stimuli.

2. In specially designed experiments in which leads were taken directly from the stimulating electrodes, an appreciable lag still occurs. In order to protect the galvanometer string in such tests, a vacuum tube "shock absorber," which limited the pick-up of shock potential to safe ranges was introduced into the circuit.[3] Figure 2D shows such a record. The local response, of course reduced in amplitude, appears on the slow decline of the residual polarization curve. In this instance the delay was about 60 msec. and comparison with leads from other points shows a definite precedence.

As is evident in the sample record of figure 2C, while a strong localized stimulus applied during late systole is capable of evoking a response, such response does not occur previous to the end of the T wave. In other words, the interval from the previous normal spike to the shock, plus the latency, approximately equals the duration of a local electrical systole. These

[3] We are indebted to Dr. Harold Green for its construction and for assistance in its experimental use.

results must be squared with apparently demonstrated facts, viz., *a*, that heart muscle is refractory during the period of depolarization, supposedly extending from the spike to the T-wave; and *b*, that the energy of the stimulus is apparently "frozen," so to speak, until the early moments of diastole. It is conceivable that repolarization begins during the so called vulnerable period; indeed, Blair et al. (1) postulated occurrence of such a phenomenon in association with the waning of contraction previous to the T wave in strips of turtle ventricle. It is conceivable that heart muscle is excitable by very strong shocks and that an impulse is then conducted very slowly. However, this would not explain the retardation of local

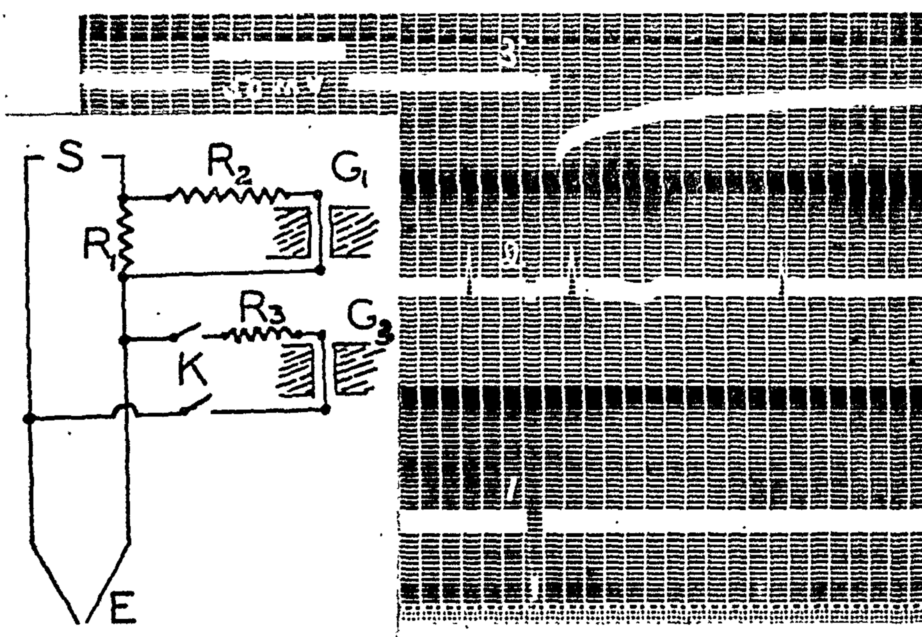

Fig. 4. Diagram of circuit used to determine magnitude and duration of polarization current resulting from strong brief D.C. shocks. Curves of lead 3 show calibration of string for 50 m.v. and by comparison that part of the polarization potential beginning about 20 msec. after the end of shock, indicated in lead 1. Time, 0.04 sec.

responses at the very point of stimulation, shown in figure 2D. Another possibility is that the tissue polarization resulting from a strong brief shock may be so great that it persists until early diastole and that the rate of decline of such polarization or its association with a sudden change in resistance during relaxation might make the waning polarization the real stimulus. In other words, while a strong shock is delivered during the refractory systole, its after-effects persisting into early diastole may be the real excitant.

In order to validate such a concept it is necessary to obtain evidence that polarization effects of such shocks last long enough and have sufficient intensity to be of excitatory value at the time of early diastole. Experi-

ments were done in which the magnitude of the polarization potential was recorded by a string galvanometer, temporarily disconnected during application of the shock. The arrangement used is shown in the diagram of figure 4. Galvanometer G_1, protected by a very high resistance, recorded the intensity of the shock. Galvanometer G_3, protected by an adequate series resistance (in this test 40,000 ohms), was disconnected briefly by an automatic circuit breaker and reconnected about 30 msec. after its application. The records, 3 and 1 of figure 4, show the electrical curves recorded by galvanometers G_3 and G_1; record 2 is a regular punctate lead by contiguous electrodes from a spot about 1 cm. distant. A calibration curve for 50 m.v. through the same resistance and electrodes is shown in an additional segment.

Analysis of such records showed that, at the time that the spike of lead 2 begins, curve 3 reveals a polarization potential of about 50 m.v. which gradually decreases to half-value in 0.08 second. By extrapolation of the exponential decline it was calculated that 20 msec. earlier, i.e., approximately at the time when the point under the stimulating electrode was stimulated—the polarization must have had a magnitude of about 100 m.v., which is probably sufficient to excite cardiac muscle during the early moments of the partially refractory phase. That this is a true polarization of tissues and not polarization of imperfect Ag-AgCl electrodes was proved by the fact that similar results were obtained after substitution of calomel-cell electrodes of special design.

Characteristics of sequential multiple deflections. Curves A, B and C of figure 5 show electrograms from two points more proximal to the source of stimulation and from one point more distal, as indicated on the accompanying diagram. They serve to illustrate reactions obtained in more comprehensive studies, too numerous to report by illustrations. They show the local potentials in various spots when a systolic D.C. shock (S) (0.02 sec.) evoked one deflection (curve A), four deflections followed by a pause and resumption of normal rhythm (curve B), and four deflections followed by true fibrillation (curve C).

The following facts stand out on careful inspection of these curves:
1. In general, the successive beats in leads 1 and 2 from points nearer the stimulated locus—even when these are on opposite ventricles—are aberrant in configuration, whereas those of lead 3 from a more distal region tend to retain their spike-like characteristic, which suggests that the latter are excited from a more nearly normal direction and probably over the regular Purkinje network.

2. The first of a multiple series of deflections (curves B and C) following a strong systolic shock is identical in form with that of a single response shown in curve A. This indicates that differences in the initial premature contraction cannot be concerned in initiation of fibrillation.

3. The four successive deflections (a, b, c, d) in either lead 2 or 3 of curves B or C differ essentially from each other in form; but corresponding beats (e.g., *b* in lead 2, curves B and C, or *b* in lead 3, curves B and C) re-

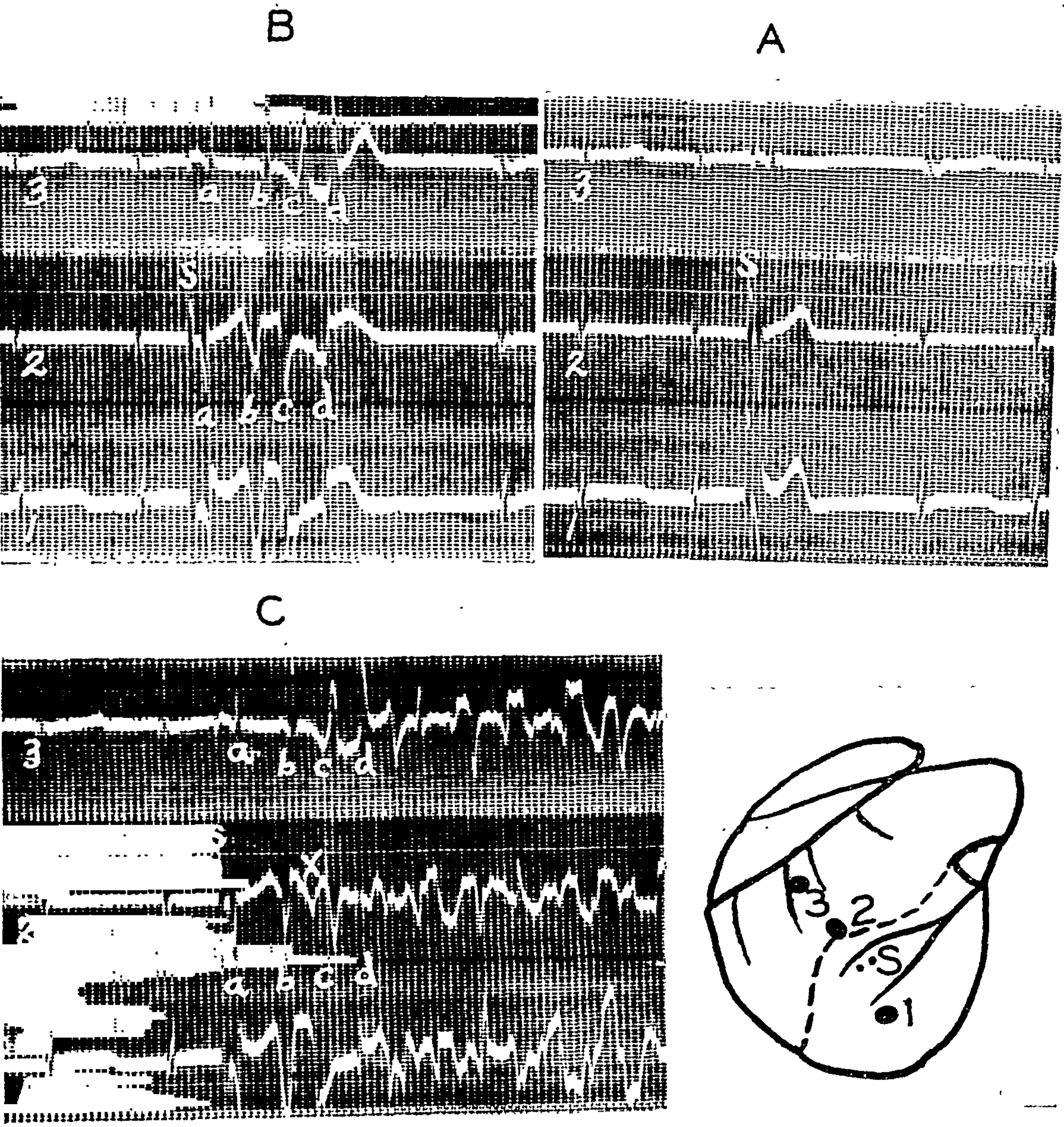

Fig 5. Three curves of punctate electrograms illustrating electrical responses to strong systolic shocks. A, a single deflection; B, multiple deflections followed by pause; C, multiple responses followed by fibrillation. Diagram shows orientation of leads *1*, *2*, *3* with respect to points of stimulation, *S*.

semble each other strikingly. This indicates that there is nothing peculiar in the nature of the second or third responses that could determine the temporary arrest or fibrillation which follows.

4. The second and third deflections in all leads are spaced at progressively closer intervals, but the shortening of periods is more marked in a proximal lead (2) than in the distal one (3). This will be discussed later.

5. The third deflection shows a similarity of form in leads 1 and 3 of curves B and C; but in lead 2 of curve C—nearest the locus of excitation—an inconspicuous earlier variation (x) appears which is not present in curve

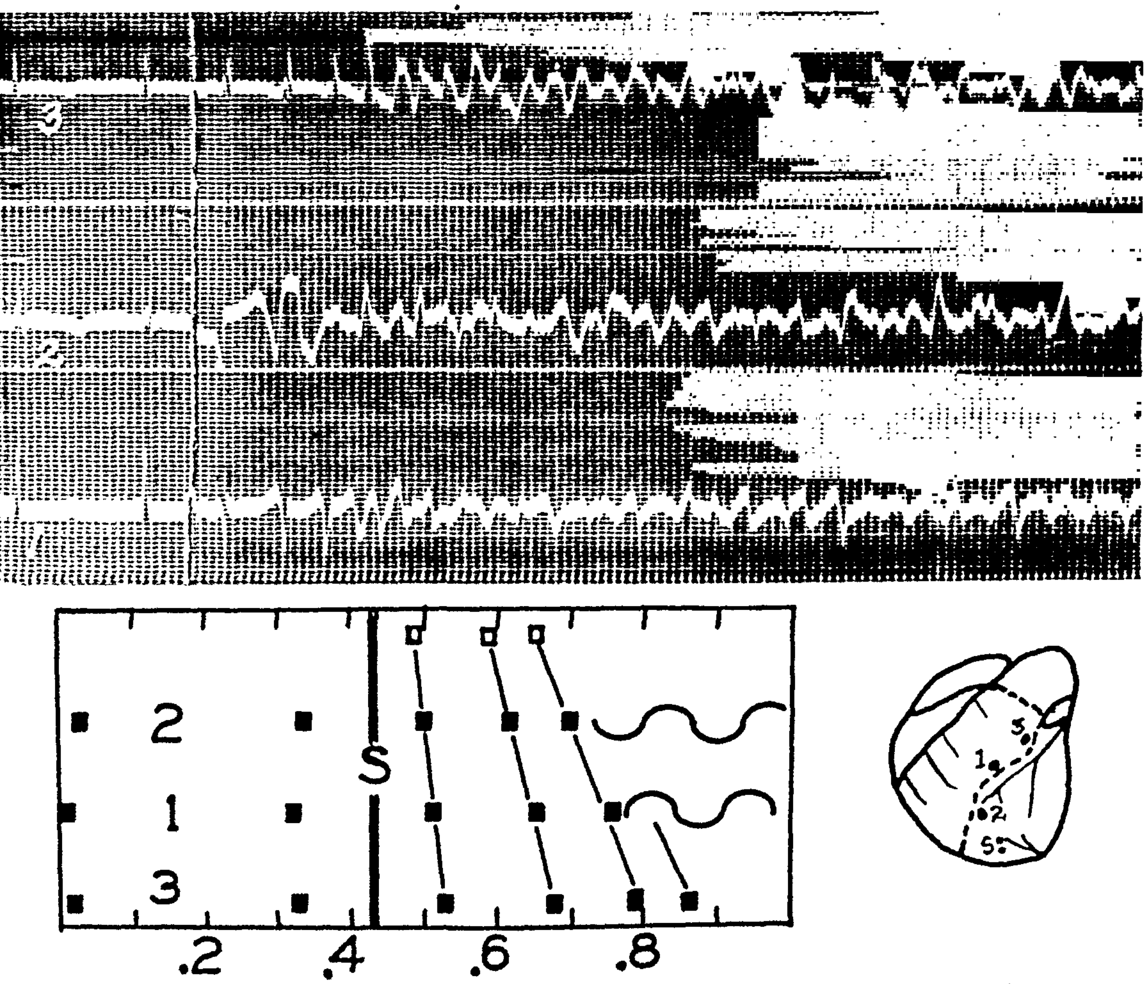

Fig. 6. Local electrograms from 3 points, as indicated in small diagram of heart, showing induction of fibrillation. Diagram illustrates interrelation of deflections and projection to a hypothetical center above. Time, 0.02 sec.

B. This indicates a disruption of rhythm at point 2 which appears to be related to a slight precedence of fibrillation at this point.

6. Despite the fact that a state of localized fibrillation starts at point 2 of curve C, the areas from which leads 1 and 3 were taken receive one additional interrelated excitation (d), just as in curve B, in which no fibrillation eventuated. The similarities in the forms and time relations of deflections *c* and *d* in leads 1 and 3 of records B and C clearly indicate that

in curve C, excitation of these areas—near and remote—are not yet due to or related to the fibrillating process started at lead 2.

The localized development of the fibrillating process. Such a localized fibrillation, which exists temporarily without involving other regions of the ventricular surface was found in a large number of instances, regard-

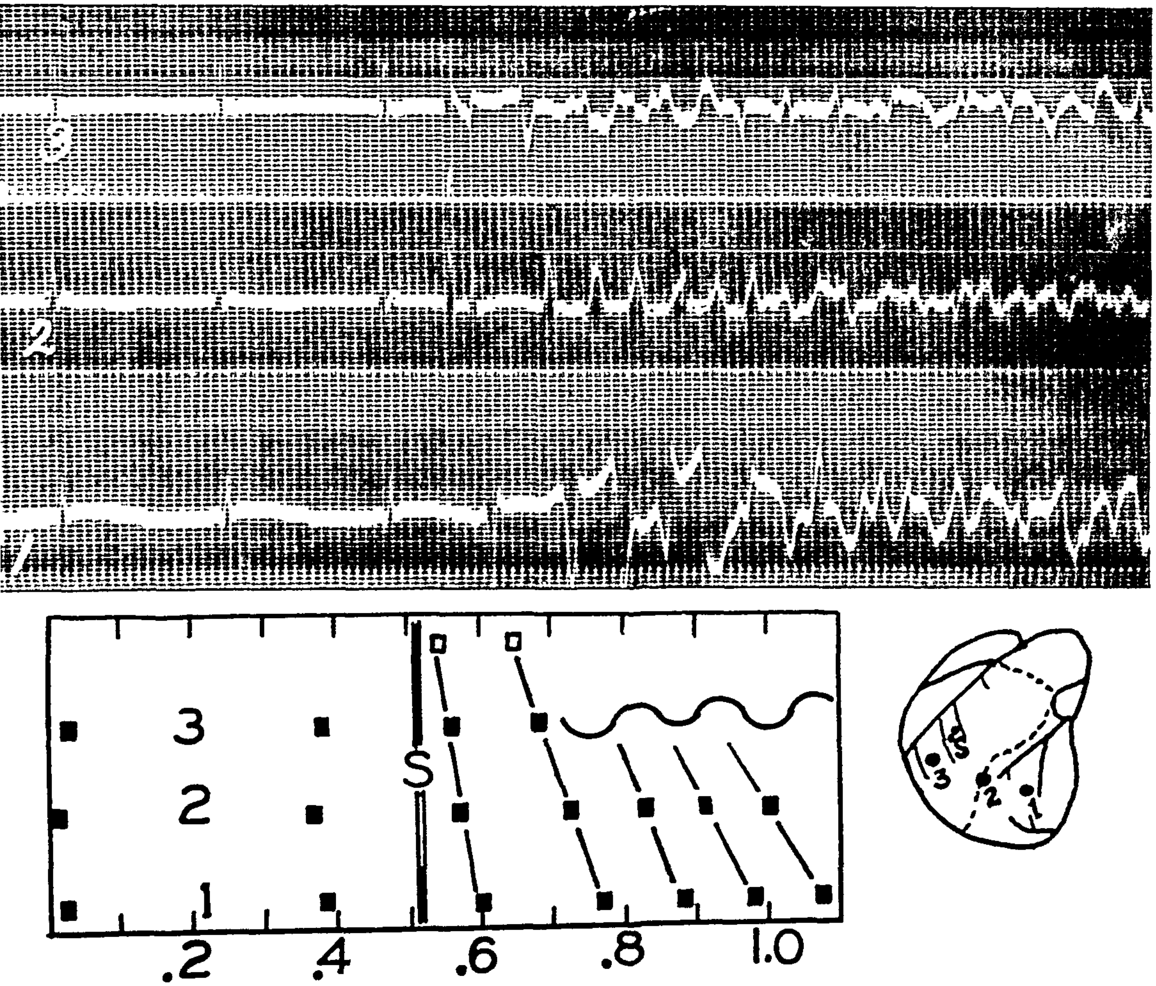

Fig. 7. Local electrograms from 3 points, as indicated in small diagram of heart, showing induction of fibrillation. Diagram illustrates interrelation of deflections and projection to a hypothetical center above. Discussion in text. Time, 0.02 sec.

less of the locus of stimulation. The fact is so important that a few additional curves are presented in confirmation.

Figure 6 shows simultaneous local electrograms from an experiment in which fibrillation was induced by a strong systolic shock near the apex. Leads 2, 1 and 3 are electrical variations recorded from points progressively more distant from the locus of stimulation, in the general direction of the interventricular septum, as indicated on the small diagram. A glance at the records indicates again that fibrillation occurs at the nearest

lead (lead 2), while at least three specific excitations at increasingly smaller intervals continue in leads 1 and 3. Such records show that there is no difference when more remote leads are taken over the septal surfaces. This has been extensively confirmed. Discussion of the plots of time relationships shown in figure 6 is advantageously deferred.

Figure 7 illustrates essentially the same phenomena following a strong systolic shock applied near lead 3 toward the base of the right ventricle. The disposition of leads was in the general direction of the superficial sinospiral bundle. We notice that the lead from area 3 nearest the point of stimulation breaks into incoördinate wavelets first. Curiously, the most distant lead 1 exhibits the greatest changes in form, while a septal point 2 lead more nearly retains the spike-like deflections and perhaps breaks into incoördinate wavelets a little later. This illustrates a type of experiment in which it appears probable that more remote areas do not continue to be excited from the initial focus, but rather from a slowly advancing fibrillating front.

Numerous experiments in which the three leads were placed along surface bundles failed to show, as in this case, that the fibrillating process necessarily spreads in the direction of surface bundles. This is not surprising in view of the complex intertwining of conduction pathways of normal and Purkinje fibers which exists in the myocardium. Such observations disprove the possibility that development of fibrillation is contingent on passage of repeated long reëntrant waves over both ventricles by common fasciculi of fibers.

Cause of the multiple accelerating beats. The intimate relation of the multiple accelerating beats to fibrillation demands the most searching inquiry possible as to their cause and the part they play in the breakdown of an orderly excitation in an initially restricted area. Two causes can be thought of to account for the preliminary undulatory movements of fibrillation which are reflected in the localized electrograms we have been describing: (1) With the exception of the first, which is clearly a premature systole, they may represent a series of reëntrant impulses, or (2), they may be due to repetitive discharges from an area around the excited points.

The first suggestion was rather favored by one of us (6), partly on observations of the surface movements in films; partly on the basis of the diminishing spacing and continually changing forms of standard electrocardiograms. If conduction rates and refractory periods of cardiac muscle established by previous investigators are accepted, such a circuit would have to be a relatively large one, and some portion of the myocardium would have to be excited every moment after the first response.

The best method for detecting such circuits would be to take numerous surface leads simultaneously during the occurrence of the second and third beats. However, evidence should be obtainable in repeated fibrillations

showing the same general character of waves in which records are taken by the only three leads available to us. Such experiments have been carried out and surface plots of the results made. An example is shown in figure 8 in which the incidence of surface negativity after a single diastolic response and the first three cycles of multiple responses to a strong systolic shock are plotted separately. Lines have been drawn at intervals representing 10 msec. differences in time of excitation. Such *isochrons* in every case have a roughly concentric configuration about a stimulated locus. *They must not be regarded as a chart of the actual spread of the excitatory wave front but rather as a chart of the sequence of surface activation, perhaps over deeper routes.*

An inspection of the four diagrams reveals that the pattern of spread of the first response following a strong systolic shock does not differ essentially from that of a single response to a diastolic shock. In both, the order of excitation is radial, in all directions from the stimulated area. The other diagrams indicate that successive responses of a train likewise radiate from the stimulated locus, often as far as the fourth deflection. This favors the view that all originate at or near the site of stimulation.

The distances between the isochrons of figure 8 indicate that, after allowing for the greater gross latency, the first response following a systolic shock reaches identical surface points at approximately the same time as diastolic shocks (cf. drawings A and B). Since the configuration of complexes is also essentially the same in the two cases, it may be assumed that the impulses traveled over the same routes and that, therefore, the conduction time is approximately the same. Since the route of travel is unknown, estimates of conduction rates are, of course, hazardous. In the case of the 2nd or 3rd beat of multiple complexes, shown in C and D of figure 8, the impulses arrive at the surface more and more slowly. This may denote an actual decrease in rate of propagation, which is in accord with knowledge that the conduction rate decreases with shortening pauses; but such a conclusion cannot be validated by our observations owing to the significant changes in contour of successive complexes analyzed in figure 5, which indicate that the different pathways were probably followed. However, regardless of the cause of the progressive delay in activation of surface points, its existence cannot be disputed.

Such plots are difficult to reconcile with the idea that the 2nd or 3rd initial complexes are due to reëntry of impulses. In many records of such beats, followed by fibrillation or not, we have never seen sequential surface changes that would fit the idea of long circuits of reëntry. The only conceivable way in which reëntrant waves could give rise to such isochrons would be to postulate that the reëntrant wave returned each time by way of deeper tissues, thus preventing its detection by surface leads. This possibility is much reduced by experiments in which internal leads, taken ap-

proximately opposite to the external ones, showed no such time lag as would be demanded if the interior represented the return path.

If the 2nd and 3rd beats represent a reëntry, a unilateral block must be produced by the shock and we would expect to find spots of delayed excitation to one side of the stimulated region in our numerous curves, at least occasionally. None have ever been found. Furthermore, according to the reëntry hypothesis, some portion of the myocardium must be excited

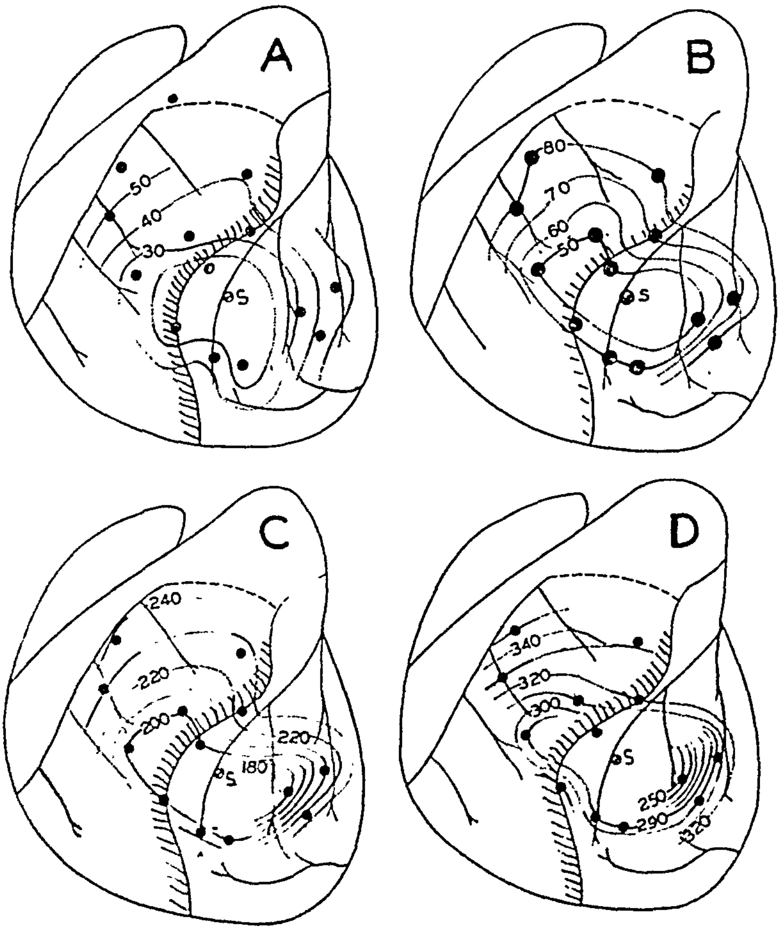

Fig. 8. Diagram showing isochrons of responses to a single response, A, to a diastolic shock, and the first three cycles (B, C, D) of multiple responses following a strong systolic shock.

at all times. Our numerous observations on the ventral surface and anterior wall always had a gap in which no point was excited. Thus the latest time following excitation at which any portion of the anterior surface was excited in figure 8B was 80 msec., and the earliest moment after excitation that the surface again became negative was 180 msec. (fig. 8C). It is difficult to believe that excited spots could have all been missed during the 100 msec. which intervened. Similarly, a gap of quiescence equal to 80

msec. intervenes between the plots of figure 8C and D. Hence, our conclusion that no portion of the heart was excited during this interval. Finally, if we retreat to the view that a small localized zigzag system develops in the vicinity of the locus of stimulation, it must have been less than the distance to the nearest electrode. This would require an inconceivable degree of slowing of conduction.

Since the analysis thus far indicates *a*, that the time of activation of concentric points around the stimulated locus increases progressively in succeeding beats; *b*, that no clear evidence of circus rings exists in the early deflections which precede fibrillation, and *c*, that the gross latency of response of concentric fields for each cycle increases systematically away from the point of stimulation, it appears improbable that reëntry of impulses can explain the 1st, 2nd and, occasionally, the 3rd and 4th beats which lead to fibrillation. It rather favors the view that they are due to repetitive firing of an area near the point of stimulation.

In the two diagrams of figures 6 and 7 respectively a crude attempt is made to illustrate the manner in which this might operate up to the time when fibrillation develops at each more distant spot. The interrelationships of spikes in the three leads while the myocardium is normally excited is indicated by the first two sets of small black squares. The time of the shock is denoted by S; and for each lead the time interval at which excitation manifests itself is indicated by another small black square. Lead 2 is nearest the locus; lead 3, farthest away. A glance at such a graph—even better than the curve itself—shows that the responses are spaced more closely at the adjacent than at the most distant point. Since the lead from the nearest point was 8–10 mm. from the stimulated locus, the latter must have discharged still more rapidly. This can be pictured by drawing slanting lines through the plotted squares and extending them to some arbitrary line indicated by white squares at the top of the graph. They represent the still more rapid tempo of the discharging area. The sloping lines, themselves, denote the progressive delay of impulses on surface points. Such a scheme helps to emphasize the idea that the beats preceding fibrillation are due to repetitive firing of impulses from a center, each one of which is transmitted more slowly to various muscle fractions. It also visualizes the common precedence of fibrillation at proximal points, while more distant ones continue to receive the retarded impulses. Admittedly, such a state of localized fibrillation is very temporary, but its brief existence is nevertheless important in analyzing the mechanism by which fibrillation starts.

The effects of accelerated induction shocks. One fact chiefly requires to be harmonized with the concept that several repeated impulses are discharged from a center, viz., the changing configuration of the successive deflections. They certainly suggest that the impulses reach the con-

tiguous electrodes from different directions, and hence that conduction disturbances are present at the same time. These disturbances may be

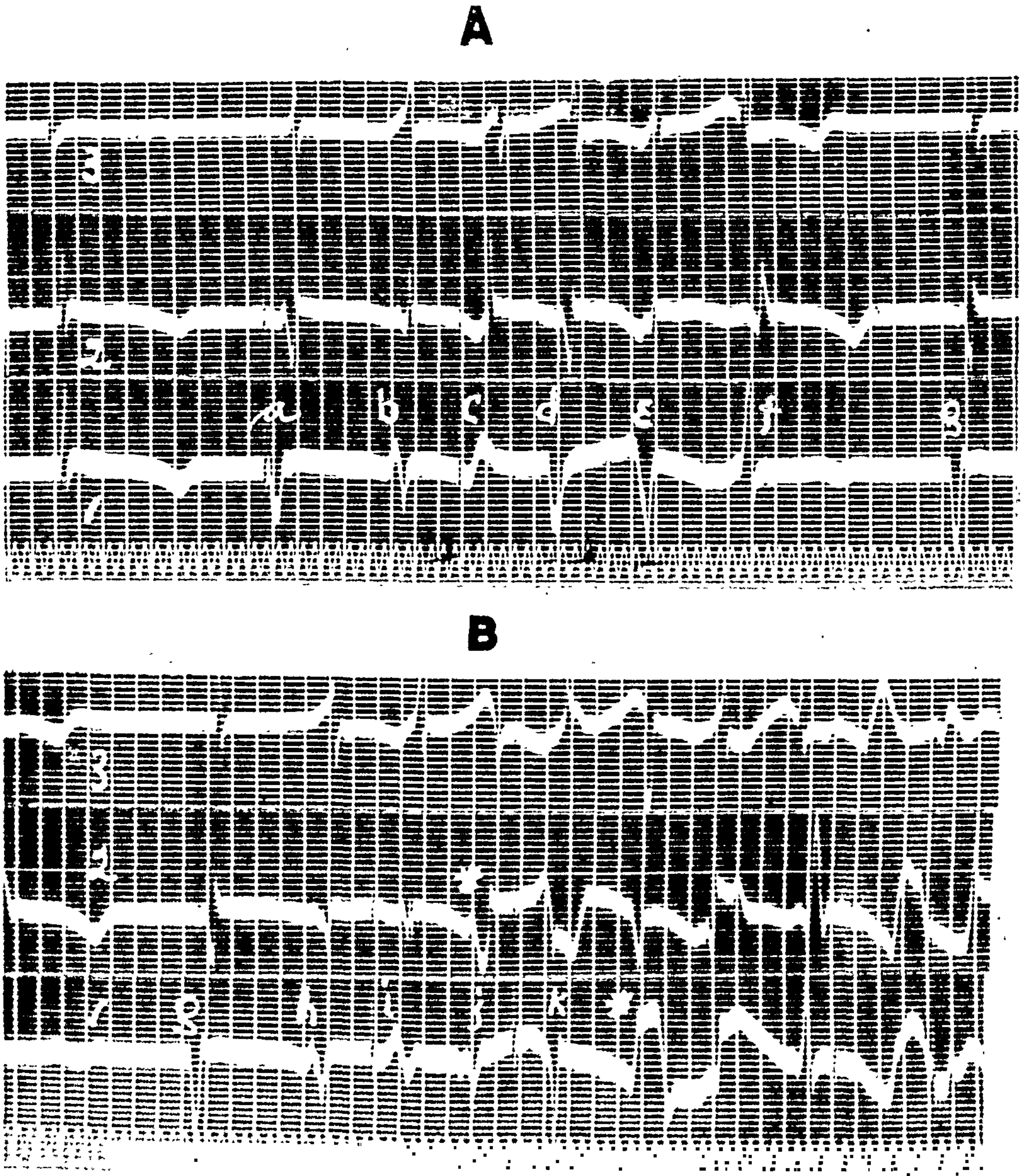

Fig. 9. Curves of preliminary electrographic deflections and fibrillation produced by an accelerating series of threshold induction shocks. A, no fibrillation, only aberrant deflections (*a*–*e*) changing from cycle to cycle; B, similar deflections (*h*–*k*) degenerating to re-entrant beats at *. Fibrillation precedes in lead 2. Time, 0.01 sec.

physiological consequences of the rapid repetitive impulses; or they may be due to a physical influence of the D.C. shock *per se*.

Furthermore, if our suspicion is correct that fibrillation develops as a

result of 2 to 4 progressively retarded impulses fired from a common center, it should be possible to reduplicate similar initial deflections and to produce fibrillation by applying to an area an artificial series of stimuli, approximately of threshold value.

Accordingly, experiments were designed in which five weak break induction shocks (makes short-circuited) were applied at diminishing intervals during a long diastole. This was accomplished by using as key a disc with five cams spaced progressively closer, each one operating a set of keys. The speed of the camshaft determined the actual intervals.

Experiments showed that a spacing of the accelerating stimuli resembling the rhythm produced by one strong D.C. shock could be achieved by trials. When such shocks are applied too far apart or come too close together fibrillation is not invoked; when properly spaced it follows regularly.

Figure 9 shows two illustrations. In A, a set of five threshold shocks is followed by a series of deflections in lead 1, nearest the stimulated area, each of which has a different configuration. This demonstrates that the conduction disturbances are associated with the rapidly repeated excitations and are not due to a direct effect of the current *per se*. In this record, the series marked *a*, *b*, *c*, *d*, *e* is followed by a spontaneous beat (f). Before a natural rhythm could be restored another artificial series of threshold break shocks were applied. The continuation of the record—in which deflection (g) is repeated—is shown as curve *B*. Four bizarre deflections (h–k) occur which are followed by fibrillation, indicated in leads 1 and 2 by an asterisk (*). The broad abnormal character of deflections due to reëntry is well shown.

Discussion. *A theory of the initiation of ventricular fibrillation.* We accept the demonstration of Garrey (4) that the incoördinate state of contraction to which the term "fibrillation" should be restricted is due to circus excitation. This is universally preceded, however, by a run of tachysystolic beats which hold the secret as to the mechanism of onset of fibrillation. The observation—important in itself—that a brief strong shock must coincide with the vulnerable period of late systole or very early diastole in order to produce such tachysystolic beats and fibrillation is not itself an answer to the question as to why it does so. We must know how a stimulus falling during a supposedly refractory phase can stimulate at all, and we must understand the physiological process it sets up which quickly makes reëntry and circulation of impulses possible.

We have presented evidence that a late systolic shock is really effective because it creates a tissue polarization of sufficient strength and duration to excite at the very first moments of the relatively refractory period. Moreover, it appears to start a rhythmic center from which several impulses are discharged at an accelerating rate, the limit of the interval being about 80 msec. These successive stimuli arrive at more remote regions, later

and later. The progressive decrease in refractory periods associated with the accelerating rate of responses, combined with delay in conduction, furnish ideal conditions for reëntry of impulses, *but only after the second, third or fourth truly premature beats have run their course.* Since myocardial fractions nearest the stimulated locus are excited at shorter intervals than more distal ones, their refractory period decreases most, and reëntry occurs there first. This explains why localized areas of fibrillation exist temporarily near the site of stimulation, while more remote ones temporarily receive periodic coördinated excitations.

Without prejudice to the view that primary alterations in conduction may account for the onset of ventricular fibrillation under other circumstances, our results do not support the view that immediate changes in the duration of the refractory period or of conduction time are responsible for fibrillation produced by a brief strong D.C. shock. On the contrary, such changes are *physiological consequences* of a rapid accelerating discharge of impulses from a center near the stimulus. The bearing of these findings on the interpretation of "spontaneous fibrillation," e.g., after coronary occlusion becomes evident on reflection.

A generalized state of fibrillation quickly develops as a result of either of two mechanisms: 1, numerous areas may start independent fibrillating centers in rapid sequence by the same mechanism and then merge, or 2, the fibrillation spreads from the initially fibrillating area to adjacent ones by reëxciting fractions as soon as they have passed out of their refractory phase. Our analysis of many records indicates that the former is relatively uncommon; the latter, usual.

SUMMARY

The mechanism by which ventricular fibrillation develops as a result of a strong, brief D.C. shock delivered during the vulnerable period of the ventricular cycle was studied electrographically. Three pairs of contiguous electrodes operating on the principle of Garten differential electrodes were variously oriented on the ventricular surface with respect to the site of stimulation in different tests on the same heart. Since the ventricles were repeatedly revived by the countershock method of Hooker, the ventricular surface was sampled reasonably well by changing the placement of electrodes.

The following results and conclusions are discussed:

1. A moderately strong, brief shock applied considerably before the T wave of an electrogram recorded at, or near, the point of stimulation causes one response shortly after the T wave. Tests are presented which indicate that the response is probably not due to an actual systolic excitation but to the creation in tissues of a decrementing polarization potential which is sufficient in duration and intensity to excite early in the next relatively

refractory phase. In this way, the response of the ventricle to a systolic shock can be harmonized with the existence of a refractory state in muscle fractions. It also explains why the latency of responses at definite points on the cardiac surface remains constant for shocks applied at any moment of diastole, but increases linearly as they are applied more and more in advance of the T wave of a local electrogram. No evidence has been found in the dog's heart that changes in conduction are involved in such increasing latency; the interpunctal differences between excitation lines remain the same, regardless of when a stimulus is introduced.

2. A very strong shock applied to a discrete mass of ventricular muscle during the Q-T interval of an adjacent electrogram evokes a series of deflections in all electrograms led from the cardiac surface. This may be followed by a pause and resumption of normal rhythm or by fibrillation. When the latter occurs, it develops in the nearest surface lead slightly before it does in the others.

3. The series of discrete deflections recur at progressively decreasing intervals in the most proximal lead and the excitation times more distant in relation to proximal points increase progressively during the series of responses.

4. The order of excitation on the surface and interior of the ventricle during the second, third and fourth responses and the existence of a period between beats when no area is excited do not support the view that the initial beats are due to reëntry of impulses. On the contrary, the concentric arrangement of isochrons calculated from actual records strongly suggests repetitive emission of several impulses from the stimulated area, temporarily made automatic by the shock.

5. Deflections similar in form and sequence in near and far areas of the heart could be caused artificially by applying a series of weak threshold break induction shocks at slightly diminishing intervals. If given in proper sequence, these also cause fibrillation.

6. The conclusion is drawn that the reëntry of impulses with which fibrillation following localized application of a strong, brief D.C. shock starts, is due to the progressive decrease in refractory period combined with a progressive increase in conduction time. This starts in regions near the site of stimulation and occurs as a result of the repetitive accelerating discharges rather than as an effect of the current *per se*. In short, while repetitive discharges from a center or centers are not required to sustain fibrillation, they are essential to its initiation after a strong electrical shock.

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THE RELATIONSHIP OF RENAL BLOOD PRESSURE AND BLOOD FLOW TO THE PRODUCTION OF EXPERIMENTAL HYPERTENSION[1]

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It appears well established (1, 2, 3) that radical alterations in the normal hemodynamics of the kidney may produce experimental hypertension. It has been demonstrated (4, 5) that in all probability such an altered kidney produces a pressor substance de novo or an increase in the amount of pressor substance normally secreted into the blood stream. As a matter of fact, a substance has been found (6) in the venous blood leaving the partially ischemic kidney of the dog, which effected a marked and rapid pressor response when given to another dog. This same substance may be neutralized, however, by the intact, functioning kidney. In its chemical and physiological properties, this particular substance was thought to be similar in certain respects to both purified renin and angiotonin (7), but not identical to either.

Despite the above observations, the initiating factor in the production or increased production of this renal pressor substance has not been clearly demonstrated. Constriction of the renal artery by a Goldblatt clamp can conceivably effect only two hemodynamic changes in the kidney, namely, 1, a reduction in the renal blood flow, or 2, a reduction in the renal blood pressure, including the pulse pressure, distal to the clamp. In the majority of clamp applications, probably both of these changes occur. Recently, however, there have been several observations recorded (8, 9, 10) which suggest that the kidney in experimental hypertension need not be ischemic. Furthermore, renal blood flow determinations performed upon human patients suffering with essential hypertension (11, 12) demonstrate that renal ischemia is not an invariable finding in this syndrome. Even in many of the cases in which a renal ischemia is present, it is frequently of so slight a degree that a legitimate doubt is justified as to whether the ischemia was the causative factor in the maintenance of the hypertension. Finally,

[1] We are indebted to the Winthrop Chemical Company for their kindness in supplying us with generous quantities of diodrast.

it may be mentioned that the detection of renin in the blood leaving the perfused kidney (13) is dependent upon a reduction in the pulse pressure rather than in the rate of blood flow to this same kidney.

In the present communication, evidence is presented which indicates that renal ischemia is neither the initiating, the causative, nor the maintaining factor in the production of experimental hypertension.

I. The renal circulation before, during, and after a constriction of the aorta above the aortic orifices of both renal arteries (*acute*). It has been recognized that the renal blood flow may remain unchanged despite alterations in the arterial pressure (14, 15, 16), but the intra-renal causes for this are not definitely known. Accordingly, the renal blood flow, the glomerular filtration rate, and the filtration fraction were observed before, during, and after a constriction of the aorta above the orifices of both renal arteries.

Methods. Six normal dogs (series A) were anesthetized with pentobarbital sodium. A left lumbar incision was made, the peritoneum incised, and the aorta above both renal arteries exposed and encircled by a loose tape ligature, the two ends of which were conducted to the outside through a brass tube 2.5 mm. in diameter. The wound was then closed. During this manipulation the kidneys were not disturbed. The left femoral artery was cannulated and connected to a mercury manometer, allowing continuous blood pressure recording. The left femoral vein was connected to an infusion flask, and for an hour following the application of the aortic ligature, the animal slowly received an infusion of normal saline solution (150 cc.).

After preliminary intravenous administration of diodrast (0.5 per cent) and inulin (1.66 per cent) in saline solution for 30 minutes, the renal blood flow, the inulin clearance, the mean femoral blood pressure and in some cases the femoral pulse pressure were obtained during a 30 minute control period. The ligature was then tightened until a decrease of 30 to 40 mm. Hg was observed in the femoral artery pressure and this degree of reduction was maintained for 40 minutes. During this constriction period, the above determinations were repeated after an initial delay of 10 minutes. The third and final determinations were made in a 30 minute period following the release of the aortic constriction.

In five other dogs (series B) exactly the same procedure was followed except that the aortic ligature was tightened until a fall of 40 to 60 mm. Hg in the mean femoral artery pressure occurred.

For the determination of the renal blood flow and the inulin clearance of each 30 minute period, three catheterized urine collections (each of 10 min. duration) and two blood samples were taken. These were then analyzed separately for iodine (according to the method of White, 17) and for inulin (according to the method of Alving, 18). The mean of these three determinations was taken as the average period value. The diodrast

plasma clearance in cubic centimeters per minute plus the addition of the volume of red blood cells (determined by hematocrit) was taken as the renal blood flow. The factor advocated by White (19) was not used in the calculation of the renal blood flow because our interest was primarily in comparative values. The filtration fraction was obtained as the inulin plasma clearance/diodrast plasma clearance.

The aortic and renal artery hemodynamics below the constriction were studied by blood pressure determinations obtained from the femoral artery. It was thought that, although there might be slight differences in the actual pressure values in these three sites, the determination of the pressure values, particularly changes, in the femoral artery afforded a reasonably accurate picture of the intra-aortic and intra-renal artery hemodynamics. Accordingly, the aortic and renal artery pressure values cited in this communication were those actually found in the femoral artery. The phasic blood pressure determinations were obtained with the Hamilton apparatus.

At the end of each experiment the animal was autopsied, the position of the aortic ligature relative to the renal artery orifices was checked, and the kidneys weighed.

Results. In the dogs of series A (see table 1-A) it was observed that although a reduction in the aortic mean pressure of 30 to 40 mm. Hg below the constriction led to a marked decrease in the pulse pressure at the expense of the systolic phase of the blood pressure, there was no significant change in the renal blood flow as measured by the diodrast clearance. In three of the six dogs there was a slight increase in flow, in the remaining three a slight decrease with an average reduction in flow of 1.5 per cent, which was within the experimental error. In all dogs a reduction in the glomerular filtration, as measured by the inulin clearance, was observed. The average decrease was 20 per cent. This fall in glomerular filtration rate resulted in a concomitant fall in the filtration fraction (see table 1-A).

The maintenance of a normal renal blood flow in these dogs during aortic constriction, despite a moderate reduction in the mean pressure and a severe decrease in the pulse pressure of the aorta and renal artery, indicated that renal vascular dilatation must have occurred. The average decrease of 20 per cent in the glomerular filtration rate indicates that there must have been a reduction in the filtration pressure within the glomerulus. The exact site of this renal vascular dilatation could not be definitely determined although the decrease in glomerular filtration despite a maintenance of a normal blood flow suggested that a dilatation chiefly involving the glomerular efferent arterioles had occurred. It is likely that the afferent arterioles also dilated, but certainly to a lesser degree. In preliminary experiments it was found that if constriction were maintained over 30 minutes a decrease in the renal blood flow uniformly occurred. Even during the 30 minute constriction period, the last 10 minute determination

of the renal blood flow was usually less than that obtained during the first 10 minutes.

After the release of the aortic constriction the mean renal artery and pulse pressure in each dog returned to a value as high as or higher than the pre-constriction value. The inulin clearance also returned to about the

TABLE 1

The renal circulation before, during, and after a constriction in the aorta above both renal arteries

EXPERIMENT NUMBER	BEFORE CONSTRICTION					DURING CONSTRICTION					AFTER RELEASE OF CONSTRICTION				
	Mean femoral pressure	Femoral pulse pressure*	Renal blood flow†	Renal inulin clearance‡	Filtration fraction	Mean femoral pressure	Femoral pulse pressure	Renal blood flow	Renal inulin clearance	Filtration fraction	Mean femoral pressure	Femoral pulse pressure	Renal blood flow	Renal inulin clearance	Filtration fraction
A. Constriction causing reduction of 30–40 mm. Hg in femoral pressure															
	mm. Hg	*mm. Hg*			*per cent*	*mm. Hg*				*per cent*	*mm. Hg*	*mm. Hg*			*per cent*
3	115		2.74	0.57	38.0	78		2.45	0.47	32.2	120		2.38	0.60	46.9
4	110		3.69	0.74	19.9	85		4.56	0.67	21.0	132		3.06	0.74	31.5
8	120	50	3.70	0.74	30.9	85	15	4.53	0.59	23.3	145	75	3.07	0.74	37.8
9	122	75	3.32	0.52	25.7	85	15	3.16	0.48	24.5	135	70	2.93	0.42	22.9
13a	100	40	3.93	0.74	14.7	62	20	1.49	0.46	24.2	100	40	2.38	0.71	23.2
14a	140		4.08	0.56	24.4	100		5.00	0.45	15.9	140		3.22	0.41	20.8
Average	118	55	3.58	0.65	25.6	82.5	16.66	3.53	0.52	23.5	128	67	2.84	0.60	30.5
B. Constriction causing reduction of 40–60 mm. Hg in femoral pressure															
2	110		2.60	0.81	31.4	65		2.51	0.66	27.7	105		1.87	0.65	35.0
5	150	60	2.92	0.60	28.0	100	10	2.98	0.57	27.6	135	55	1.85	0.59	56.2
6	155		4.30	0.78	28.9	100		2.94	0.61	32.1	160		2.69	0.78	45.4
7	135	65	3.02	0.63	34.2	82	10	2.78	0.53	31.2	135	60	3.38	0.89	43.9
10	140		3.73	0.57	22.6	86		2.94	0.44	22.1	110		2.73	0.48	26.5
Average	138	62.5	3.31	0.68	29.0	87	10	2.83	0.56	27.9	129	57.5	2.50	0.68	41.4

* Obtained with the Hamilton Blood Pressure Apparatus.

† Calculated as the diodrast clearance in cubic centimeters per minute per gram of kidney divided by the hematocrit plasma volume percentage.

‡ Calculated as the inulin clearance in cubic centimeters per minute per gram of kidney tissue.

pre-constriction clearance, but the renal blood flow sharply decreased (average reduction, 20 per cent). This comparative ischemia, despite the normal arterial pressure and glomerular filtration rate, strongly suggested that an increase in the tonus of the glomerular efferent arteriole had occurred. For it was difficult to observe any other change in the kidney

circulation which had taken place that could have reduced the rate of flow and not the rate of glomerular filtration. This ischemia, however, was usually temporary and disappeared at the end of an hour.

In the dogs of series B, it was found that when the mean aortic pressure was reduced 40 to 60 mm. Hg, there was not only a decrease in the pulse pressure (average reduction, 84 per cent), and in the inulin clearance (average reduction, 16.5 per cent), but also in the blood flow (average reduction, 14.5 per cent). The filtration fraction likewise showed a slight decrease (average reduction, 4 per cent). Aortic constriction of this severity, then, decreased not only the pulse pressure and the glomerular filtration rate, but also the renal blood flow. As already observed in the dogs of series A, the release of the constriction was followed by a return of the inulin clearance and the pressure to about pre-constriction levels. The renal blood flow, however, decreased even more (average reduction, 24 per cent) after release of the constriction than had been observed in the dogs of series A. The filtration fraction (average filtration fraction, 41.4 per cent) thus became even higher and represented a 43 per cent increase over that found during the control period. Here again, the return of all factors studied to the pre-constriction level with the exception of the renal blood flow pointed to the probability of the occurrence of increased glomerular efferent arteriolar tonus.

II. The renal circulation before, during, and after a constriction of the aorta above the orifice of the left, but below the orifice of the right renal artery (acute). Methods. Six dogs were studied. Exactly the same procedure was carried out as previously described in dogs of series B (40–60 mm. Hg reduction in mean aortic pressure), except that the ligature was placed around the aorta above the left renal, but below the right renal artery aortic orifice. By this procedure, then, one kidney with normal hemodynamics was present in a dog whose remaining kidney was subjected to arterial pressure changes. Preliminary determinations of carotid artery pressures prior to and following the aortic constriction revealed no consistent changes in aortic pressure above the constriction.

Results. As table 2 clearly demonstrates, although the effects of aortic constriction upon one kidney gave total renal changes roughly parallel to those found previously in dogs of series B, except for a lesser degree of renal ischemia, a return of the renal blood flow to normal, together with an actual decrease in the inulin clearance and filtration fraction, occurred after the release of the constriction. These last observations suggest that the increased filtration fractions observed in dogs of series A and B were not due to neurogenic or mechanical factors, but were due in all probability to the elaboration of a humoral substance, apparently formed during the period of low arterial and pulse pressure in the *absence of renal ischemia* (see table 1-A). In this last series of dogs, this substance was probably

neutralized by the normal kidney present for, as has been mentioned (6), the normal kidney appears to neutralize quickly the effect of the pressor substance present in the blood leaving a kidney whose artery has been severely clamped.

III. The renal circulation and the systemic blood pressure, before, during, and after a reduction in the aortic blood pressure (acute and chronic). The above observations strongly suggested that a kidney subjected to a moderate reduction in its mean pressure and a severe reduction in its pulse pressure nevertheless could maintain a normal renal blood flow for a short period of time and that during this period it apparently produces a substance capable of causing glomerular efferent arteriolar spasm. However, no immediate systemic hypertension was observed in any experiment. It was thought advisable therefore to determine whether this substance apparently formed by a non-ischemic kidney under reduced pressure (mean and pulse) would later produce a chronic hypertension.

TABLE 2

The renal circulation before, during, and after a constriction in the aorta above the left, but below the right renal artery

EXPERIMENT NUMBER	BEFORE CONSTRICTION					DURING CONSTRICTION					AFTER RELEASE OF CONSTRICTION				
	Mean femoral pressure	Femoral pulse pressure	Renal blood flow*	Renal inulin clearance†	Filtration fraction	Mean femoral pressure	Femoral pulse pressure	Renal blood flow	Renal inulin clearance	Filtration fraction	Mean femoral pressure	Femoral pulse pressure	Renal blood flow	Renal inulin clearance	Filtration fraction
	mm. Hg	*mm. Hg*			*per cent*	*mm. Hg*	*mm. Hg*			*per cent*	*mm. Hg*	*mm. Hg*			*per cent*
1b	125		3.94	0.77	34.0	82		3.72	0.57	23.8	110		2.8	0.44	27.2
2b	135		3.36	0.69	29.9	85		3.62	0.73	28.2	135		4.0	0.73	26.6
3b	125	55	3.66	0.58	23.8	82	15	3.72	0.36	14.5	132	65	3.93	0.61	23.3
4b	125		3.29	0.59	27.0	85		2.55	0.42	25.0	120		4.03	0.59	23.3
5b	125	35	4.7	0.62	34.4	85	8	3.39	0.49	35.0	125	37	4.0	0.42	26.7
6b	130	35	4.13	0.54	22.0	85	10	2.96	0.42	24.1	85	30	3.5	0.36	17.4
Average	128	42	3.85	0.63	28.5	84	11	3.32	0.49	25.1	118	44	3.71	0.53	23.9

* Calculated as the diodrast clearance in cubic centimeters per minute per gram of kidney divided by the hematocrit plasma volume percentage.

† Calculated as the inulin clearance in cubic centimeters per minute per gram of kidney tissue.

Methods. Five uninephrectomized dogs, previously trained for blood pressure determinations, were used. Uninephrectomized dogs were used in order to avoid the extensive surgical manipulation associated with the application of the aortic clamp above two renal arteries. A preliminary renal denervation was also performed on one of the five dogs (4-c).

After stable blood pressure levels had been reached, the dogs were anesthetized with pentobarbital sodium, the aorta was exposed as before and encircled by a silver clamp, although no compression of the aorta was effected at this time. The clamp itself was similar to the Goldblatt designed clamp, except considerably larger and equipped with a rectangular screw head, over which a tightly fitting rectangular tube extending to the outside could be fitted. The clamp and tube having been connected, the field was quickly closed, and the tube allowed to project from the wound. After one hour the renal blood flow, inulin clearance and blood pressure (femoral) were determined over a 30 minute period as control values under anesthesia. A needle attached to a mercury manometer

TABLE 3

The effect of a constriction of the aorta upon the femoral blood pressure and the renal circulation (acute and chronic)

EXPERIMENT NUMBER	CONTROL VALUES					ACUTE EFFECT OF COARCTATION				CHRONIC EFFECT OF COARCTATION				
	Control blood pressure*	Control blood pressure under anesthesia	Renal blood flow†	Renal inulin clearance‡	Filtration fraction	Femoral blood pressure	Renal blood flow	Renal inulin clearance	Filtration fraction	Maximal femoral blood pressure after constriction	Duration of hypertension	Renal blood flow (72 hrs. post aortic constriction)	Renal inulin clearance (72 hrs. post aortic constriction)	Filtration fraction (72 hrs. post aortic constriction)
	mm. Hg	*mm. Hg*			*per cent*	*mm. Hg*			*per cent*	*mm. Hg*	*days*			*per cent*
1c	135/100	140/110	354	94.2	36.3	110/100	326	63.5	27.6	155/130	7			
2c	125/85	130/90	205	42.5	36.3	105/95	247	43.5	32.1	138/118	14			
3c	120/90	130/90	236	54.0	34.2	80/75	205	45.3	33.1	150/140	14	210	51.5	36.8
4c§	140/85	140/95	164	28.6	26.7	105/95	158.5	22.2	21.4	130/120	12	173	41.5	37.0
5c	130/80	140/95	221	52.4	32.4	105/95	323.0	38.2	16.9	105/95	0	312	72.0	33.9
Average	130/90	136/96	236	54.3	33.1	101/92	251.9	42.5	26.2	135/121				

* All blood pressures obtained from right femoral artery with Hamilton Blood Pressure Apparatus.

† Calculated as the diodrast clearance in cubic centimeters per minute per square meter of surface area divided by the hematocrit plasma volume percentage.

‡ Calculated as the inulin clearance in cubic centimeters per minute per square meter of surface area.

§ Kidney previously denervated.

was inserted into the femoral artery and the tube connected to the aortic clamp was turned until there was a reduction of 20 to 30 mm. Hg in the mean femoral pressure. After 10 minutes a second renal blood flow and inulin clearance determination was performed over a 30 minute interval. The tube was then disengaged from the aortic clamp by means of a plunger insertion, allowing the clamp to remain encircling and compressing the aorta. The femoral blood pressure of the five animals was followed daily, and in three of the five dogs a repeat renal blood flow and inulin clearance determination was performed 72 hours after aortic constriction. The renal blood flow and inulin clearance values, adjusted to one square meter of surface area, are given in table 3.

Results. As table 3 indicates, this degree of aortic constriction affected specifically the systolic phase of the blood pressure causing an average decrease of 77.5 per cent in the pulse pressure. Despite the fall in the mean and pulse pressure, it will be observed that the average renal blood flow in the five dogs showed a slight increase (average increase, 6 per cent) following the reduction in the renal blood pressure by aortic constriction, although this increase was not considered significant. The inulin clearance and filtration fraction fell immediately after the constriction as had already been observed in the acute experiments. But when these five dogs were allowed to survive, it was found that four of them developed a chronic hypertension (see table 3), usually manifested as a rise in the diastolic pressure one to two days following the application of the clamp. The production of hypertension has been reported (20) to occur following constriction of the aorta above the renal arteries, and Steele (21, 22) pointed out that in this type of hypertension the diastolic increased more than the systolic phase, if the pressure were obtained from the femoral artery.

In three of these chronic dogs, the diodrast and inulin clearances were repeated and, although two of these dogs (3-c, 4-c) were hypertensive at the time of the determinations, there was no significant renal ischemia present in the three dogs examined. There was, however, an increase in the inulin clearance in two, and an increase in the filtration fraction in all three.

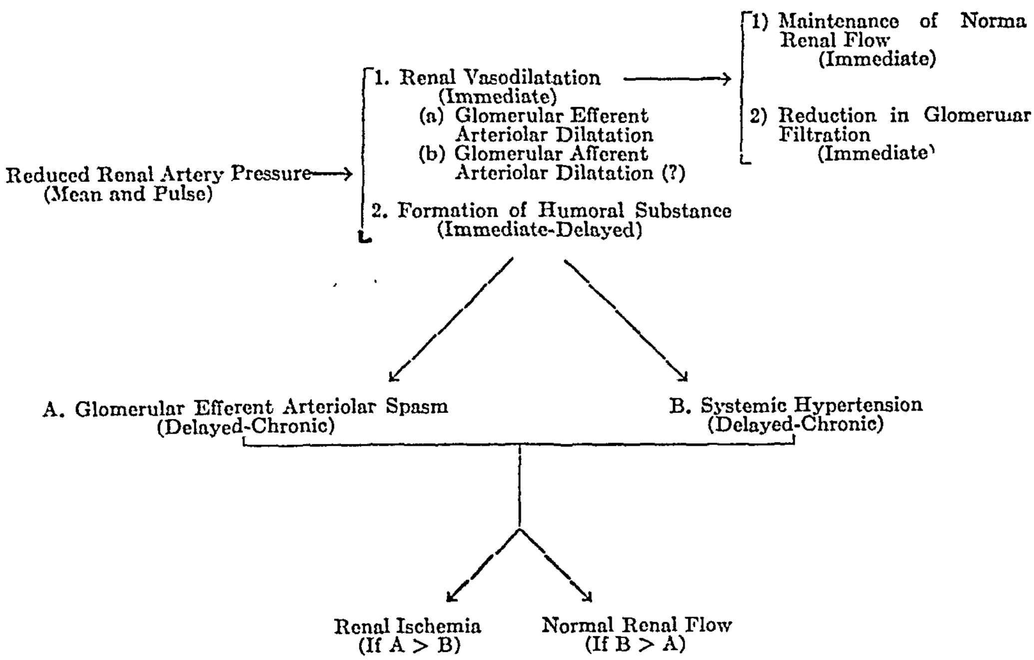
Discussion. The observations obtained from the experiments described above, indicate that a reduction in the renal artery pressure (both mean and pulse), is followed by a renal vasodilatation, and a fall in the glomerular filtration rate. If the fall in pressure is not too great, there will be no immediate ischemia. During this period of reduced renal artery pressure and glomerular filtration rate, a humoral substance, capable of neutralization by a kidney with normal hemodynamics, appears to be formed. This substance in turn apparently effects a glomerular efferent arteriolar spasm causing a delayed ischemia which occurs even after the release of the aortic constriction. It is important to point out that renal ischemia does not appear necessary for the production of this substance.

Hypertension was produced in four out of five dogs by moderate constriction of the aorta above the renal artery orifice. However, no ischemia was observed either immediately after the constriction of the aorta or three days later when a systemic hypertension was present. *From these observations it appears that renal ischemia is neither the initiating nor the maintaining factor in experimental hypertension.*

In all the dogs, however, there was evidence of increased glomerular efferent arteriolar spasm, and it was believed that the renal ischemia occur-

ring secondarily in the acute experiments was absent in the chronic experiments because of the rise in the systemic blood pressure in the hypertensive dogs. In other words, it is highly probable that renal ischemia in experimental hypertension of this type is not only a secondary phenomenon, but may even be absent if the systemic pressure rises high enough to overcome the increased glomerular efferent arteriolar spasm produced by the humoral substance of the deranged kidney. Considered from this viewpoint, the presence or absence of renal ischemia in experimental hypertension is dependent upon the ratio of the amount of glomerular efferent arteriolar spasm to the intensity of the systemic hypertension. The following diagram illustrates the probable changes occurring in experimental hypertension as suggested by our results.

DIAGRAM 1



SUMMARY AND CONCLUSIONS

1. The renal hemodynamics and systemic blood pressure were studied following aortic constriction above and between the renal artery aortic orifices.

2. It was found that renal ischemia is not necessary for the initiation or maintenance of a chronic (renal) experimental hypertension.

Note: The authors wish to express their thanks to P. Blakesley, H. L. Newman, E. MacNaughton and J. Smith for their aid in the execution of this problem.

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THE EFFECT OF DESOXYCORTICOSTERONE ACETATE AND OF BLOOD SERUM TRANSFUSIONS UPON THE CIRCULATION OF THE ADRENALECTOMIZED DOG[1]

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I. *The Effect of Desoxycorticosterone Acetate upon Blood Pressure and Plasma Volume.* Potent adrenal cortical extracts, even in large amounts, will not induce either a transient or persistent elevation in blood pressure above normal levels in man or animals (1). Within recent years, however, several clinical investigators have reported that the synthetic adrenal hormone D.C.A. (desoxycorticosterone acetate) may cause hypertension in the Addison's disease patient (2, 3, 4, 5). Elevations in pressure have also been observed in normal dogs and rats following its use (1, 6). Since the Addison's disease patient on D.C.A. therapy also shows a striking increase in plasma volume (2, 3, 4), the elevation of pressure might be assumed to be dependent in some manner upon the volume rise. The first section of the present study is concerned with this possible relation between blood volume and pressure changes in adrenalectomized and intact dogs given D.C.A.

Blood pressures were determined by the intra-arterial needle puncture method (7) without anesthesia, all animals being table trained. Plasma volumes were measured by the blue dye T-1824 (8). The dogs were fed a constant diet of Ken-L-Ration with a supplement of 2 grams NaCl. With this dietary regime, the maintenance dose of D.C.A.[2] was from 0.25 to 0.5 mgm. per dog per day, in the interval between experiments.

A. *Blood pressure.* Daily injections of D.C.A. induced, after a lag period of about 48 hours, a slow but progressive rise in arterial pressure, reaching a peak within 6 to 13 days (table 1, fig. 1). With continued treatment, the pressure might show a slight regression or be maintained at this peak level. In all cases it stabilized at some 20 to 30 mm. Hg above the normal.

[1] Part of the expenses of this investigation were defrayed by Julian M. Livingston of New Rochelle, N. Y.

[2] The desoxycorticosterone (percorten) used in these experiments was supplied through the generosity of Ciba Pharmaceutical Products, Inc.

When cortical extract was substituted, the pressure slowly declined to normal or near normal within 7 to 10 days.

Of seven adrenalectomized dogs studied, we have observed but one which failed to show this elevation in blood pressure (dog 7, table 1). One other animal, observed through four continuous cycles of D.C.A. therapy and cortical extract substitution, failed to show a significant rise in pressure on the third cycle, but did show a full rise later (fig. 3). With the possible

TABLE 1

Blood pressure and plasma volume changes in adrenalectomized and intact dogs placed on D.C.A. therapy

DOG	ON CORTICAL EXTRACT			ON D.C.A.					
	Plasma volume	Plasma volume	Blood pressure	Dosage	Plasma volume	Plasma volume	Time to vol. peak	Blood pressure	Time to pressure peak
Adrenalectomized dogs									
	cc.	*cc. per kgm.*	*mm. Hg*	*mgm.*	*cc.*	*cc. per kgm.*	*days*	*mm. Hg*	*days*
1	490	46.2	102	0.5	591	54.7	4	126	10
2	420	46.2	100	0.5	588	60.6	4	120	6
3	721	55.9	104	1	848	61.4	4	129	13
4	500	50.0	100	1	799	74.0	6	147	10
5	459	52.5	100	2	630	70.8	5	122	8
6	568	44.4	104	5	732	55.0	6	118	8
7	597	55.3	105	5	656	60.2	7	110	13
Ave.	536	50.1	102		692	62.4	5	124	10
Intact dogs									
1	659	46.4	109	2	708	52.8	12	128	12
2	614	57.4	111	2	701	60.9	10	120	10
3	485	54.8	112	4	552	67.3	14	120	14
4	621	56.5	108	5	632	60.0	8	108	
Ave.	595	53.8	110		648	60.2		119	

exception of this latter animal, a positive correlation between D.C.A. dosage and the extent of the pressure rise was not observed (table 1).

The dog with intact adrenal glands, given equivalent daily doses of D.C.A., also shows a definite tendency toward an elevated blood pressure, but the change was neither as marked nor as regular as in the adrenalectomized animals (table 1 and fig. 2).

B. *Plasma volume.* The adrenalectomized dog placed on D.C.A. therapy showed a plasma volume gain within the first 24 to 28 hours. A definite peak was reached in 3 to 7 days, after which, despite the continuance of D.C.A. therapy, the volume usually declined, to stabilize at 5 to 10 cc.

per kgm. body weight above the normal (fig. 1). An occasional dog has shown a spontaneous decline to normal after 20 to 30 days of D.C.A. therapy. The plasma volume changes have not appeared to be necessarily correlated with the dosage employed.

The intact dogs were apparently not as sensitive to D.C.A. as the dog lacking adrenals in so far as plasma volume increases are concerned (fig. 2). Two of four dogs studied showed plasma volume increases, while the others revealed no change despite prolonged treatment with relatively

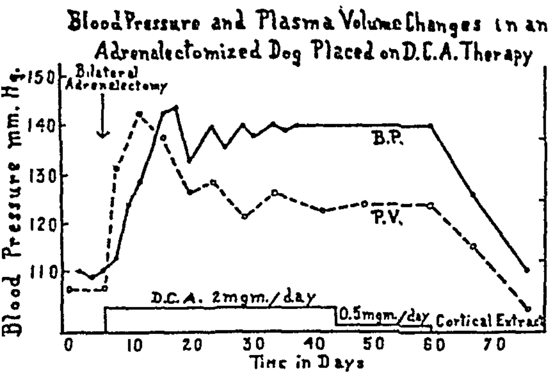

Fig. 1

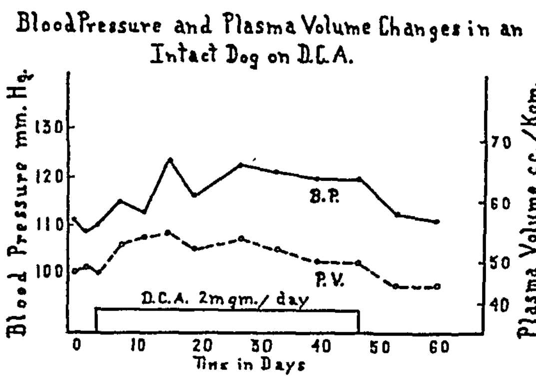

Fig. 2

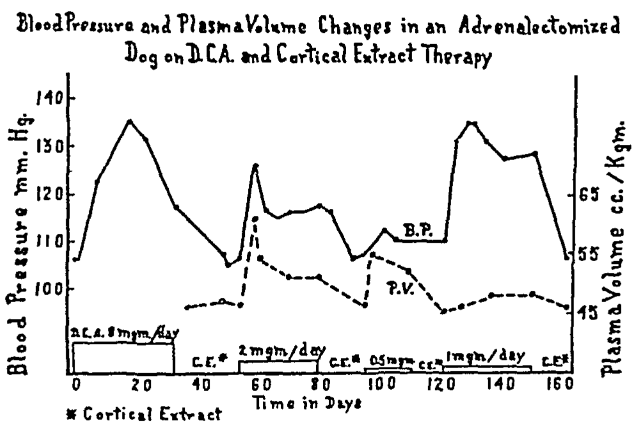

Fig. 3

large doses. One of the two dogs which failed to respond, was later adrenalectomized, and treated continually with a D.C.A. dosage of 5 mgm. per day. In spite of this high dosage, still no change in volume was observed (dog 4, table 1).

It might be assumed that the persistent elevation in blood pressure which follows use of D.C.A. therapy was dependent upon the rise in plasma volume. That this is not necessarily true is indicated by several facts: 1, the plasma volume rise always precedes the pressure rise; 2, by the time the peak of the pressure rise has been attained, the plasma volume has

significantly declined; 3, the plasma volume may increase without an accompanying pressure rise, and we have also observed dogs which exhibited a well marked pressure rise without significant change in the plasma volume (fig. 3, cycles 3–4); 4, in those cases where D.C.A. therapy is long continued, the plasma volume may decline to normal while the pressure remains high; 5, when cortical extract is substituted for D.C.A., the plasma volume has invariably returned to normal, while the pressure may remain somewhat above normal. It would seem, therefore, that the factors responsible for the persistent high blood pressure are more complex than a simple dependence of the blood pressure upon the plasma volume.

Grollman, Harrison and Williams (1) have suggested that the hypertensive effect of D.C.A. may be due to a toxic action of steroids on the kidney. This idea is not supported by these experiments, however. 1. The adrenalectomized dog receiving D.C.A. usually shows a return to normal blood pressure levels when cortical extract is substituted. Study of electrolyte concentrations, blood volume, and hemoconcentration, have revealed that the pressure fall was not due to inadequate extract dosage. Return to normal pressure levels was observed in all except one dog, and the length of time the animal had been receiving D.C.A. and the number of times the pressure had been elevated, seemed not to affect the outcome (fig. 3). 2. The intact dog exhibits a smaller response to D.C.A. than does the adrenalectomized animal. 3. In the face of a possible renal damage, one might perhaps expect a rise in blood urea nitrogen with D.C.A. therapy, whereas the reverse is usually true. It would seem that kidney damage is not of primary importance in producing the persistent elevation of pressure.

II. *The Effect of Transfusing Blood Serum from Normal Dogs into Adrenalectomized Animals.* The writers have repeatedly called attention to certain disabilities of the peripheral circulation of adrenalectomized dogs not receiving cortical hormone therapy. Among other changes, these animals appear to show marked atony of the capillaries resulting in peripheral stagnation, pooling of blood, anoxemia and increased permeability (9, 10, 11, 12). Much of the evidence for this view has been of an indirect nature. In the following experiments, increased permeability of the capillaries of the adrenalectomized dog apparently can be readily demonstrated. The experiments reveal, in a striking manner, the effect of cortical extract and D.C.A. in restoring to normal the capillary circulation.

Sterile blood serum, collected from large normal dogs, was infused into the jugular vein at a rate of 3 to 4 cc. per minute, the temperature of the serum being maintained at 37°C. throughout. Wherever possible serum obtained from the donor animal was divided into two portions and given to an adrenalectomized and a normal control recipient. The interval between withdrawal of blood and transfusion of the serum was approximately fifty minutes. The amount of serum given was 160 cc.

A. *Adrenalectomized dogs receiving daily maintenance doses of cortical extract.* Both intact dogs, not receiving therapy of any kind and the active, vigorous, adrenalectomized animal, receiving daily maintenance doses of cortical extract show no symptoms of circulatory failure or signs of edema when given serum transfusions. A representative case of the series of adrenalectomized dogs receiving maintenance extract is shown in table 2 (dog 4). The blood pressure rose while the transfusion was in progress but declined to the normal level within two hours. Hematocrit and hemoglobin values indicated a hemodilution at the end of the transfusion, of the order to be expected if the injected serum was largely retained in the circulation. At the end of two hours both hemoglobin and hematocrit had returned to pre-transfusion levels. The evidence seems perfectly clear that both the intact dog and adrenalectomized animal receiving adequate maintenance extract do not differ in their responses to serum transfusion.

B. *Dogs showing mild adrenal insufficiency.* Six dogs were used in this study. The daily maintenance injections of cortical extract, or D.C.A., were discontinued and the animals permitted to develop mild degrees of insufficiency. The interval between the time of extract withdrawal and development of symptoms varied, but was considerably longer for those animals maintained on D.C.A. in oil for an extensive period of time than for animals receiving extract.

With the exception of dog 1, table 2, all animals were eating full rations when used as recipients for the transfused serum. When the arterial pressure had declined to 75 to 80 mm. Hg the transfusions were started. At that time the average plasma volume loss, as determined by the dye method, was 125 cc. per dog. The average loss as derived from hematocrit and hemoglobin values was 190 cc. Therefore, a transfusion of 160 cc. was used in all cases.

The animals (table 2) showed a pressure rise varying from 5 to 15 mm. Hg during the first stage of the transfusion, which usually persisted until 80 to 100 cc. of serum had been injected. In two cases, the pressure remained at the initial level or above until the end of the transfusion (table 2, dog 2). In four cases, it had declined below the starting level by the conclusion of the transfusion. Regardless of whether the arterial pressure was at the starting level or not, it invariably declined to shock levels within 2 to 7 hours. Five of six animals exhibited circulatory failure within 2 hours of the completion of the transfusion, so that it was necessary to administer cortical extract to revive them.

The transfused adrenalectomized animals all developed edema, especially marked around the eyes, lips and ears. Two animals also showed edema of the legs, abdominal wall and scrotal sacs. One dog which had previously received an injection of dye for a plasma volume determination, exhibited blue stained edematous areas scattered over the body. The

TABLE 2

The effect of transfusing normal blood serum into adrenalectomized dogs

DOG	DATE	TIME	BLOOD PRESSURE	PULSE	HEMATOCRIT	HEMOGLOBIN	REMARKS
			mm. Hg	*per minute*	*per cent*	*gm. per cent*	
Dog 1 13.4 kgm.	2/14	10:00 a.m.	114	68	32.2	13.6	Discontinued maintenance D.C.A. therapy
	2/23	3:50 p.m.	68	52	53.0	18.5	Started serum transfusion
		4:30 p.m.	56	112	56.1	19.2	Finished transfusion (160 cc.). In collapse, massive edema eyes and lips
		6:45 p.m.	52	68			Edema more severe
		11:30 p.m.	47	72			Complete collapse. Given 5 mgm. D.C.A.
	2/24	9:30 a.m.	57	72			Stronger, edema disappeared. Given 2 mgm. D.C.A. per day
	2/27	9:30 a.m.	102	92	37.0	14.7	Normal
Dog 2 13.1 kgm.	5/31	10:00 a.m.	102	80	29.0	9.1	Discontinued maintenance cortical extract
	6/5	6:00 p.m.	75	112	45.2	10.8	Started serum transfusion
		6:42 p.m.	90	96	41.8	10.2	Finished transfusion (160 cc.). One hour later dog showed progressive edema of face, neck and legs
		8:45 p.m.	46	88	46.2	11.3	Complete collapse. Marked edema. Injected cortical extract
	6/6	12:15 p.m.	75	104	34.2	9.9	All symptoms and edema disappeared. Started second serum transfusion
		12:45 p.m.	102	84	30.9	8.9	Finished transfusion (160 cc.). No symptoms
		3:00 p.m.	108	78	32.8	9.0	Normal, no symptoms or edema
Dog 3 10.7 kgm.	6/7	10:00 a.m.	104	68	44.1	13.5	Discontinued maintenance cortical extract
	6/10	3:20 p.m.	74	124	47.1	13.8	Started serum transfusion
		3:45 p.m.	87	146			Received 80 cc.
		4:00 p.m.	58	116	49.8	14.2	Finished transfusion (160 cc.). Marked edema of face, legs and scrotum
		6:00 p.m.	46	132	52.4	15.8	Very weak, marked edema. Injected cortical extract
		9:40 p.m.	82	104	48.2	14.1	Marked improvement, edema almost disappeared

TABLE 2—*Concluded*

DOG	DATE	TIME	BLOOD PRESSURE	PULSE	HEMATOCRIT	HEMOGLOBIN	REMARKS
			mm. Hg	*per minute*	*per cent*	*gm. per cent*	
Dog. 3—*Cont.*	6/11	1:45 p.m.	84	76	40.6	12.6	Edema almost gone, started second transfusion
		2:30 p.m.	88	76	50.3	13.5	Finished transfusion (160 cc.). Depressed, no further edema
		4:30 p.m.	84	100	49.0	13.5	No symptoms, active
	6/12	9:35 a.m.	98	80			Normal
Dog 4 9.5 kgm.	6/6	3:10 p.m.	112	70	30.2	8.3	On maintenance cortical extract. Started transfusion
		3:50 p.m.	122	80	23.8	6.6	Finished transfusion (160 cc.). No symptoms
		5:40 p.m.	110	76	29.9	8.1	No symptoms, no edema, normal

edema showed significant decrease within 2 hours after cortical extract was administered intravenously and, with the exception of one animal, had completely disappeared within 24 hours.

Only two dogs showed a hemodilution, slight in extent, at the end of the transfusion. The other four dogs either failed to show dilution or actually showed a blood concentration, and all dogs showed hemoconcentration two hours after the completion of the transfusion. There was no evidence that the transfused serum remained in circulation; on the contrary, the evidence all pointed to the conclusion that even much of the serum in circulation previous to the transfusion had also been lost.

Two of the transfused dogs died; the others were restored to normal health by cortical extract or D.C.A. injections when they were in collapse. The animals receiving extract made a very rapid recovery with demon strable improvement in the circulation within 2 hours. Since D.C.A. in oil, injected intramuscularly, required a longer time for absorption, the recovery of D.C.A. treated animals was less dramatic (table 2, dog 1).

C. *Transfusion of extract treated adrenalectomized dogs during recovery from circulatory collapse.* Three dogs which had received serum transfusions on the previous day, and had developed circulatory collapse necessitating administration of cortical extract, were again transfused during the recovery phase. At this time the blood pressure had risen from shock levels to about the same level to which it had fallen prior to the first transfusion (table 2, dogs 2 and 3). After the second transfusion, no signs of

circulatory embarrassment or edema appeared. The blood pressure of two animals rose to, and remained normal throughout the transfusion and thereafter, and the blood was diluted as evidenced by decreases in hemoglobin and hematocrit levels (table 2, dog 2). One animal (table 2, dog 3) of this series showed some edema still persisting from the transfusion of the previous day, when given the second serum transfusion. The animal responded to the transfusion without aggravation of the edema or evidence of strain on the circulation, although some hemoconcentration took place.

The chief point of interest is that when cortical hormone was present, even though the blood pressure was low at the beginning of the transfusion, the response of the animal was essentially similar to that of the intact dog and quite different from the response seen the day before in the same animal, when no hormone was available.

Discussion. The fact that potent cortical extract will not cause elevation of blood pressure above normal, indicates that the efficiency of D.C.A. in this respect is due to some intrinsic property of this steroid. Other steroids of the cortex are not known to produce this effect, in the dog, at any rate. However, Grollman, Harrison and Williams (1) state that the "hypertensive" effect of D.C.A. is not specific for the normal rat. Although Reichstein and Euw (13) isolated D.C.A. from cortical extracts, it is apparently present in smaller quantities than the other steroids known to possess physiological activity. The extremely low concentration of D.C.A. in crude extract may perhaps explain the inability of the latter to cause persistent elevation of the pressure above normal. The writers have elsewhere (9, 10, 11, 12) presented evidence that D.C.A. is highly effective in maintaining the functional integrity of the peripheral vasculature of the adrenalectomized dog. It apparently prevents atony of the arterioles and capillaries and seems to maintain the normal permeability of the latter. D.C.A. when used as a prophylactic fore-treatment, will prevent the circulatory failure which invariably follows various shock inducing procedures in adrenalectomized dogs, and will restore to normal the collapsed circulation once it has developed (12, 14). It seems possible, therefore, that this steroid may induce persistent "hypertension" in adrenalectomized dogs by reason of the cumulative action of several factors: 1, increase in plasma volume; 2, increase in the inherent tone of the peripheral vessels.

Evidence from the serum transfusion experiments indicates that cortical hormones are probably concerned with maintenance of normal capillary permeability. It is surprising that transfused serum should so rapidly leak through the capillaries of the adrenalectomized dog carrying with it much of the animal's own blood fluid, thereby leading to rapid circulatory failure and edema. Since such changes do not occur in the transfused intact dog, or the adrenalectomized animal receiving maintenance extract, and are readily corrected by cortical hormones, it is apparent that they

must be due to cortical hormone deficiency somehow affecting the permeability of the capillaries. However, there is always the possibility that the adrenalectomized dog not receiving extract, and without hormone reserves, is peculiarly sensitive to normal blood serum or perhaps to hypothetical substances which might form during the course of its preparation for transfusion. Menkin (15) has shown that cortical extracts, wholly or in part, will inhibit the effect of substances such as leukotaxine which increase capillary permeability in intact animals.

SUMMARY

1. Potent adrenal cortical extracts will not induce elevation of blood pressure above normal levels in either intact or adrenalectomized dogs.
2. Desoxycorticosterone acetate causes persistent elevation above normal of both blood pressure and plasma volume of the adrenalectomized dog. The effect of comparable dosage upon blood pressure and plasma volume of the intact dog is less consistent and striking.
3. There appears to be little if any correlation between D.C.A. dosage employed and extent of the blood pressure rise when doses of more than 0.5 mgm. per dog per day are used.
4. The blood pressure rise which follows D.C.A. therapy in adrenalectomized dogs, is usually associated with an increase in plasma volume, but the blood pressure elevation is not necessarily dependent upon the increased plasma volume.
5. Transfusion of blood serum of normal dogs into other normal dogs and into adrenalectomized animals receiving adequate maintenance doses of cortical extract or D.C.A. does not induce symptoms of any kind. Similar transfusions of serum into adrenalectomized dogs not receiving extract or D.C.A. therapy promptly lead to circulatory collapse accompanied by edema. Injection of extract causes disappearance of edema within a few hours and the blood pressure slowly rises to normal levels with disappearance of all symptoms.
6. Adrenalectomized dogs with lowered blood pressure can be transfused with serum without circulatory embarrassment or signs of edema if given cortical extract or D.C.A. before transfusion.
7. The experiments on serum transfusion offer supportive evidence for the view that the permeability of the capillaries is markedly increased in the adrenalectomized dog not receiving extract and that cortical hormones restore the permeability of these vessels to normal.

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THE SEQUENCE OF FRACTIONATE CONTRACTION AT DIFFERENT SURFACE REGIONS ON THE RIGHT AURICLE AND VENTRICLES OF THE DOG'S HEART[1]

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It has been recently shown that the occurrence of fractionate contraction at different regions on the surface of the dog's heart is coincident, within the limits of error of measurement, with the main peak of the differential potential-time curve recorded from the same region[2] (1). The differential curve thus offers an accurate means for mapping the sequence of onset of contraction at different surface regions. This report is concerned with the determination in this way of the sequence of onset of contractions on the surface of the right auricle and the ventricles of the dog's heart.

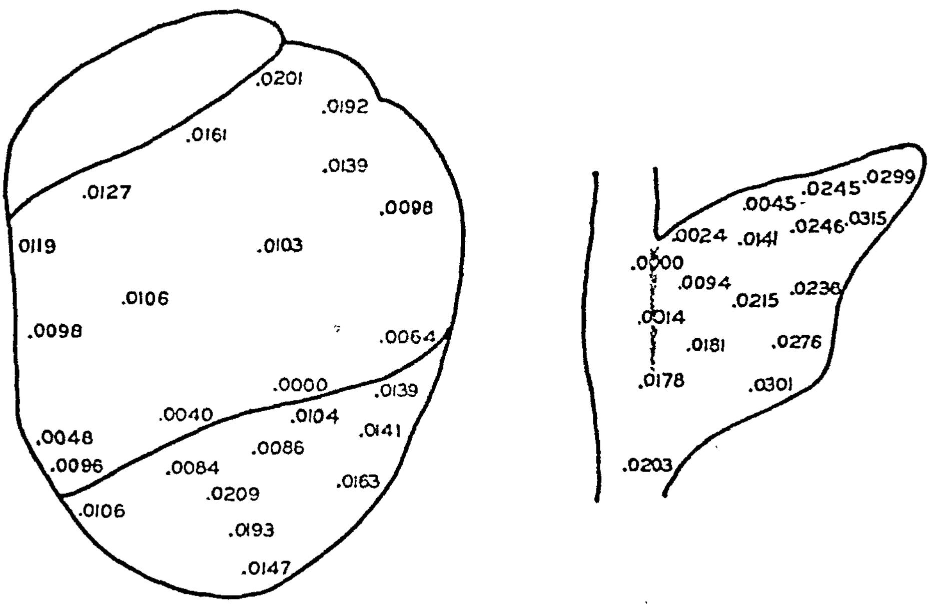
METHODS. The differential electrodes used were made up of two zinc zinc-sulphate electrodes provided with a common wick and mounted close together. The wick was held stretched in the form of a V and in contact with the heart surface by means of a thread passed through it. The electrode was connected through a direct current amplifier to a cathode ray oscillograph. A reference curve was recorded simultaneously by means of a unipolar lead, usually from the apex of the right auricle or the apex of the left ventricle, connecting to a second amplifier and oscillograph. Recording was made on 35 mm. unperforated film, driven at a speed of 110 mm. per second. Measurements were made in a comparator with 40× magnification.

RESULTS. The relative times of occurrence of the main differential peak from various surface regions on the anterior surface of the right auricle and the anterior surface of the two ventricles are given in the figure. In each case they represent the average from the data of seven experiments. Very definite differences in time of onset of fractionate contraction in different regions are apparent. The interval between the first and last regions involved is of the order of 0.03 sec. for the auricle and 0.02 sec. for the ventricles. The first regions entering into fractionate

[1] Supported in part by a grant from the Wisconsin Alumni Research Foundation.

[2] The term "fractionate contraction" was introduced by C. J. Wiggers (2) to designate local contraction of regions of heart muscle as distinguished from contraction of the chamber as a whole, as recorded by the usual suspension methods.

contraction on the surface of the right auricle are those contiguous to the upper part of the sulcus terminalis and from here contraction proceeds in rather regular fashion to the appendage and downward to the auriculo-ventricular junction. In the ventricles, the first surface contractions appear on the right ventricle contiguous to the inter-ventricular groove. Other regions enter into contraction in a manner which fails to show any evidence for a progressive involvement such as exists in the right auricle. In general, the surface of the right ventricle becomes involved before that of the left, with the exception of the conus of the pulmonary artery, which always enters into contraction late.



ANTERIOR SURFACE OF VENTRICLES

RIGHT AURICLE

Fig. 1

We have also recorded, in a few experiments, the relative time of occurrence of the main differential peaks from the posterior surface of the ventricles. These follow the same general pattern as on the anterior surface—indicating early contraction along the inter-ventricular sulcus and involvement in general of the right before the left ventricle.

Discussion. Previous work in this field has been concerned with attempts to determine an electrical state which signals the occurrence of the "impulse" of "excitation process" which presumably precedes the onset of contraction. The criterion which has been employed in nearly all of this work has been the assumption that the occurrence of "excitation" is coincident with a local fall of potential or state of "negativity." This

electrical state was supposed to spread over the muscle in the form of a "wave of negativity" coincident at all regions with the "impulse." Several different methods have been employed by workers in this field. In many cases bipolar leads from two separate regions on the heart surface have been used, in spite of the fact that the fallacies inherent in this method are obvious (3). Bipolar curves are composites of the potential changes under each electrode and it is impossible to resolve them into their two components. A few workers have used unipolar leads (4, 5), the indifferent electrode being placed on the chest wall or on a leg. The relative times of occurrence of maximum negativity at various regions were determined by reference to a constant curve, usually an electrocardiogram, recorded simultaneously. In efforts to determine the maximum state of electrical negativity in a region, the "monophasic action current," obtained from leads from an injured and an uninjured region on the heart surface, has been used (6) under the false assumption that the curve results from the development of a negative electrical state at the uninjured region (7).

The differential electrode, of the type employed by us in the present work, was first suggested and employed by Clement (8) and later by Erfmann (9). The current flow in the differential electrode is small and it was difficult to obtain satisfactory curves with the unamplified string galvanometers, which these investigators employed. The criterion used for the occurrence of excitation was apparently the start of the differential curve, but deductions as to the spread of the "wave of excitation" were made from the form of the recorded curves.

It is obvious from recent work that we have no proven criterion at present for the determination of the onset of the state of excitation, if it is assumed that this state precedes the onset of contraction. The onset of fractionate contraction in a region is however accompanied by an electrical state defined by two types of electrical curves that may be recorded, the unipolar and differential potential-time curves. The onset of contraction is coincident or nearly coincident with the occurrence of the most rapid time rate of change of potential in the region as indicated by the unipolar curve and by the maximum flow of electrical current as indicated by the maximum potential of the differential curve. In practice the latter is more easily identified and is the preferable curve for the determination of the sequence of occurrence of fractionate contraction at different regions of the heart surface. It is to be noted that the *start* of these curves is theoretically simultaneous from all parts of the heart and is found to be so provided sufficient sensitivity of the recording apparatus is employed. The occurrence of the events on these curves noted above as associated with fractionate contraction in the region occurs at different times in different regions. Presumably, the state of "excitation" in a region begins at some instant which precedes the occurrence of the maximum time rate of

potential change, as indicated by the unipolar curve and the occurrence of the maximum peak of the differential curve, but we do not at present know what point, if any, on these or any other electrical curves recorded from the heart, signals this instant. There is certainly no valid reason to associate it with maximum "negativity" or "positivity" of the region, since either state may precede or follow the onset of fractionate contraction in the region.

SUMMARY

The sequence of occurrence of fractionate contraction at the different surface regions of the right auricle and ventricles of the dog's heart is determined by recording differential potential-time curves from the various regions along with a constant reference curve. The results are indicated in the accompanying figure.

The fact that no criterion is available at present which can be used to determine the instant of "excitation" of a region of heart muscle, is brought out and discussed.

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ON THE MECHANISM OF ENHANCED DIABETES WITH INFLAMMATION[1]

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The effect of an infection or inflammation on human diabetes is well known to clinicians and pathologists. In brief, the course of the disease is considerably accentuated, and to some extent the inflammatory reaction is intensified. There is also some evidence of a generalized fall in resistance as indicated by an augmented susceptibility to infection. The mechanisms involved to explain the enhancement in the diabetic condition as well as the increased severity of the local inflammation have never been satisfactorily elucidated.

There is evidence that the healing of surgical incisions is delayed when the treatment of diabetes is inadequate. This question has been recently studied by Bennett in depancreatized dogs subsequently deprived of insulin (1, 2). The rapid course assumed by pulmonary tuberculosis in diabetic patients is common knowledge (3). Sweet has pointed out that the serum obtained from dogs in the end stages of diabetes loses some of its normal bactericidal power (4). More recently Richardson has reported that the complement in the blood of diabetic patients does not seem to differ in amount from that of the blood in non-diabetics. This has been found true irrespective of whether or not infection is present. The antibacterial power of the blood of diabetic patients seems, however, reduced. Finally this investigator has found in diabetics inoculated with typhoid vaccine a diminished capacity for agglutinin formation (5). The nutritional state of experimental animals as exemplified by depleted liver glycogen seems to be concerned with the development of a lowered agglutinative titre after administration of typhoid vaccine (6). The organs of

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depancreatized cats after cutaneous inoculation of bacteria show the presence of the microörganisms with greater frequency than the corresponding organs of normal controls (7). As a result of these various studies Richardson has concluded "that at least a part of the commonly recognized susceptibility of diabetics to infection might be concerned with this decreased power to form immune bodies as compared with normal individuals."

A recent survey of the literature by Perla and Marmorston (3) have led these authors to conclude that the hyperglycemia and the decreased sugar tolerance are apparently not the cause for the reduced antibody production. According to Wilder (2) resistance to infection is not diminished in a patient whose diabetes is well controlled and in whom nutrition is adequate. Nevertheless this author points out that a diabetic patient requires several times more insulin when infection intervenes. Rabinowitch infers that an insulin-destroying enzyme is involved, especially since pus cells are known to inactivate insulin *in vitro* (8). In this connection, Jensen, in a recent monograph, has reviewed the literature indicating that insulin can be inactivated by proteolytic enzymes (9). Greene and his collaborators (10) have recently made a comprehensive clinical study on the relation of delayed healing of clean and infected wounds to the height of the blood sugar level. They have concluded that there is no apparent connection between the height of the blood sugar level and the delayed healing of wounds or of infections in diabetes mellitus.

The present series of observations represents an attempt at elucidation of the basic mechanism concerned in explaining enhanced diabetes concomitant with inflammation. The results indicate that the excessive hyperglycemia seems referable to an increased proteolysis at the site of inflammation. The deamination of the split protein molecule favors the formation of a surplus of glucose which in turn gradually diffuses into the circulating blood stream. The enhanced local proteolysis induces severe tissue damage thus offering an explanation for the intensified degree of inflammation. The increased protein catabolism as well as the increased formation of sugar in the inflamed area can be readily controlled by administration of insulin.

Experimental. *The effect of inflammation on the blood sugar of depancreatized dogs.* An attempt was first made to reproduce as closely as possible the clinical diabetic condition with superimposed inflammation. All experiments were made on depancreatized dogs. It is, however, to be borne in mind in this connection that although there are many obvious points of similarity between the human form of the disease and this type of experimental diabetes, nevertheless there are also several notable differences. These have been recently reviewed by Long (11).

Blood samples were withdrawn from the heart of a series of dogs weighing from about 5 to 15 kgm. Blood sugar was determined by the Folin

method (12). Pancreatectomy was performed under nembutal anesthesia. All forms of nourishment with the exception of fluids in the form of skim milk or water were withheld from 12 to 24 hours following operation. After that time usually 50 grams of boiled lean horse meat mixed with 2 grams of sucrose and 2 grams of choline chloride formed for each of the two succeeding days the bulk of the solid food. Subsequently the ration of meat was gradually increased to about 150 or even as high as 300 grams per day. The sucrose was also increased to about 3 or 4 grams daily. In few instances raw pancreas was administered instead of choline chloride. Insulin administration began about 12 to 24 hours following pancreatec-

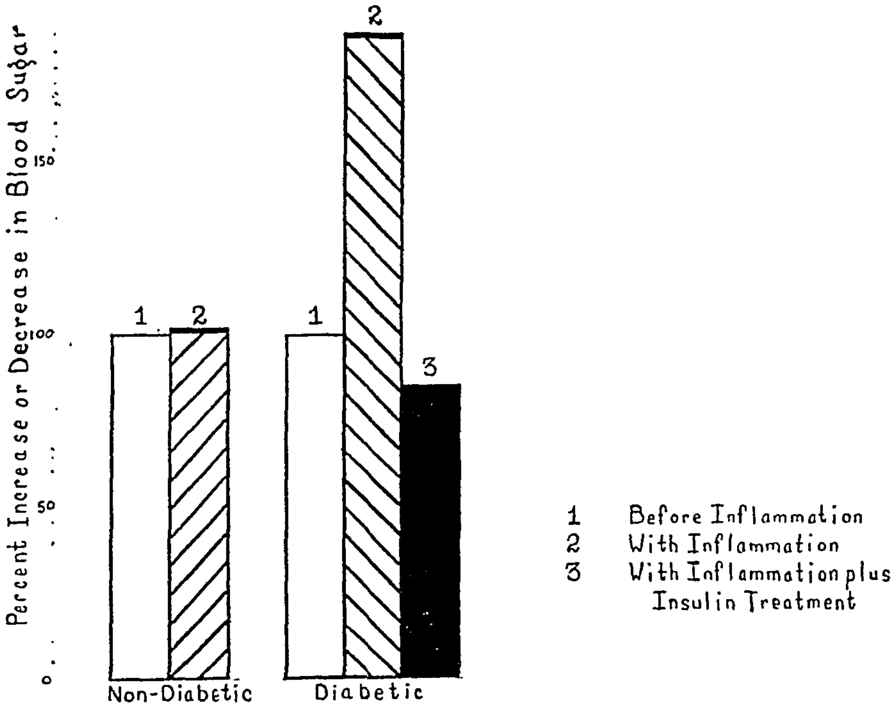

Fig. 1. Effect of inflammation on the blood sugar of non-diabetic and of diabetic dogs.

tomy. The dose of insulin consisted at first of about 3 units administered twice daily. The quantity of insulin was gradually increased to about 10 units daily. Blood sugar determinations were made almost daily. After a period, usually ranging from about 6 to 12 days, insulin administration was discontinued. After about 2 or 3 days the animal, under nembutal anesthesia, received an intrapleural injection of 1.5 cc. of turpentine. This, as described in previous studies, is a convenient method of inducing an acute inflammatory reaction accompanied by extensive exudation (13). At variable intervals, ranging from about 7 to 44 hours, blood samples were studied for their sugar content. Similar observations were made on

non-diabetic dogs in which a pleural inflammation likewise had previously been induced by the introduction of turpentine.

The results of all observations are summarized in table 1 and graphically illustrated in figure 1. It is clear that with a superimposed and extensive inflammatory reaction involving the whole of the right pleural cavity there occurs a sharp ascent in the blood sugar level. The average blood sugar in depancreatized animals is 253.0 mgm. per 100 cc. prior to the injection of the irritant. Following the injection of the irritant there is a rapid and sharp rise averaging 469.1 mgm. This is an average increase in blood sugar of 85.4 per cent. With administration of insulin throughout the period of the experiment, as will be pointed out later on, this rise is wholly inhibited (fig. 1).

TABLE 1

The effect of inflammation on the blood sugar of diabetic and non-diabetic dogs

NUMBER		DURATION OF INFLAMMATION		DIABETIC DOGS		NON-DIABETIC DOGS	
Diabetic dogs	Non-diabetic dogs	Diabetic dog	Non-diabetic dog	Blood sugar prior to the introduction of the irritant	Blood sugar subsequent to the injection of the irritant	Blood sugar prior to the introduction of the irritant	Blood sugar subsequent to the injection of the irritant
		hrs.:mins.	*hrs.:mins.*	*mgm./100 cc.*	*mgm./100 cc.*	*mgm./100 cc.*	*mgm./100 cc.*
1	9	7:20	6:23	311.7	497.3	120.3	95.9
2	9	9:20	24:50	224.1	269.2		118.1
3	10	15:45	10:40	257.1	522.2	112.9	83.8
4	11	24:30	23:00	240.9	412.4	71.4	74.8
5	12	25:15	23:10	163.3	312.8	69.8	55.6
6	13	25:18	24:45	257.4	630.9	81.7	111.1
7	14	33:32	24:50	335.3	442.9	63.7	64.2
8	15	14:00	25:05	234.0	482.0	69.3	70.8
8	16	44:15	42:45		652.5		108.1
	17*		6:15			81.0	57.2
Average				253.0	469.1	83.8	84.0

* Splenectomized two days prior to injection of irritant.

On the other hand, control non-diabetic animals with pleural inflammation fail to show any rise in blood sugar (table 1, fig. 1). Splenectomy was performed in one instance (see dog 17, table 1) to control for the operative procedure involved in pancreatectomy. The blood sugar level likewise failed to show any rise following the intrapleural injection of the irritant.

In figure 2 the rapid rise in blood sugar following the introduction of an inflammatory irritant in a depancreatized animal is compared with the course of the blood sugar level in a diabetic animal without any in-

duced pleurisy. It is clear that in the latter the blood sugar, though elevated, never reaches the hyperglycemic level encountered in a diabetic dog with a concomitant and severe inflammation.

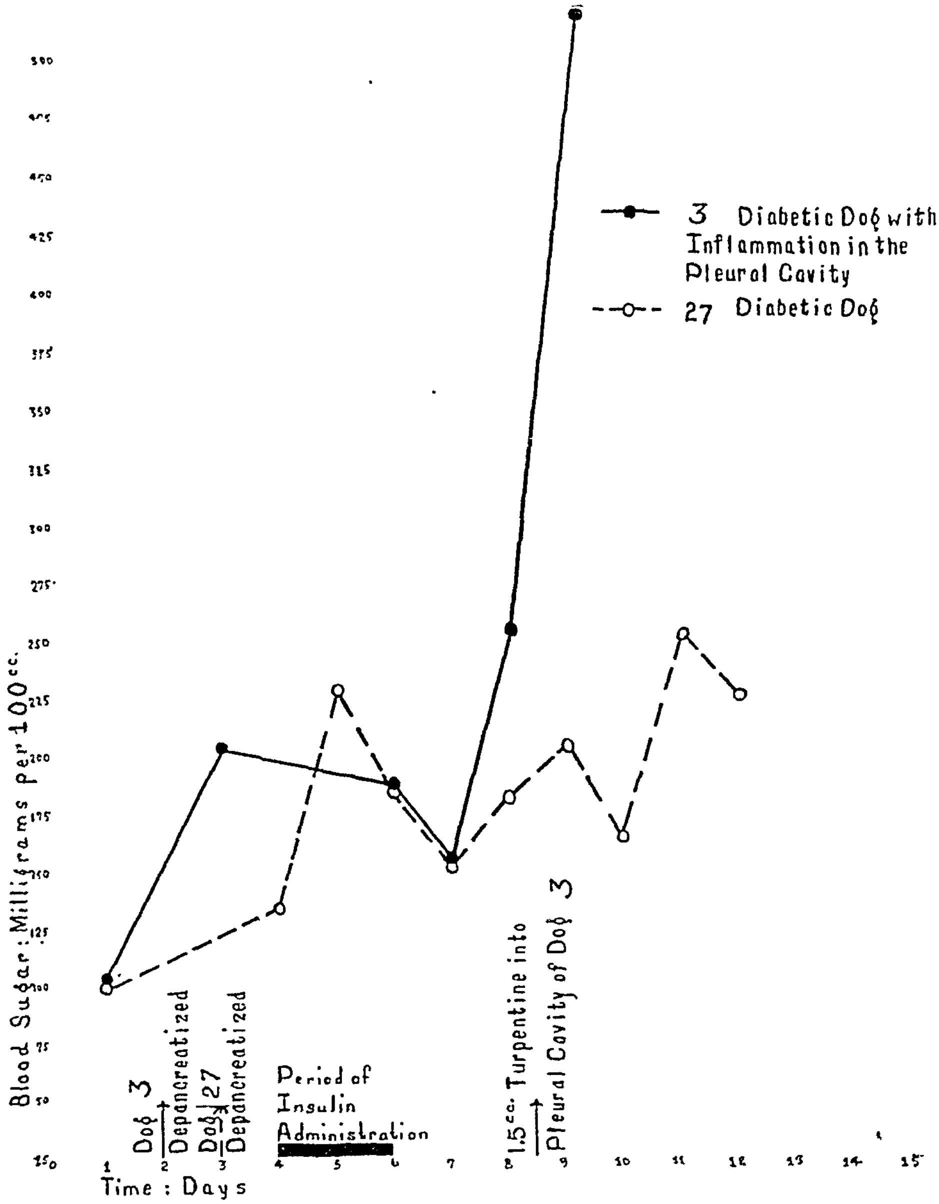

Fig. 2. Effect of inflammation on the blood sugar of a diabetic dog

These results seem to duplicate quite well the change in blood sugar level which one observes in a diabetic patient whose clinical course is complicated by infection.

Carbohydrate and protein metabolism in exudates of depancreatized dogs. What is the basic mechanism which induces a sharp rise in the blood sugar of diabetic dogs having a superimposed pleural inflammation? There

is one important feature of inflammation which must first of all be considered. One of the cardinal signs of this important manifestation of cellular injury, besides the other well-known criteria observed and described by the early writers, is the phenomenon of proteolysis (14).

The occurrence of protein digestion in inflammatory exudates has been known for a long time. The products of proteolytic digestion in exudates were recognized by Eichwald in 1864, who reported what he thought was a peptone in pus. Friedrich Müller (15) described the autolytic property of purulent exudates in tuberculosis and pneumonia. Opie (16) studied thoroughly the enzymatic property of cells of an exudate in digesting coagulated protein. According to this author the ability of phagocytic cells to remove injurious material is dependent on the possession of proteolytic enzymes. The importance of proteolysis in inflammation was again stressed by the writer in his recent studies on the isolation of leukotaxine (14, 17). By comparing both the amino acid nitrogen and the total proteins of exudates with the concentrations of these same constituents in the blood serum, he concluded that proteolysis forms a conspicuous feature of the inflammatory reaction.

The formation of glycogen from part of the protein molecule has been known for a long time. Claude Bernard believed in the possibility of such a conversion. The subsequent work of numerous investigators attested further the truth of this belief. The experiments of Wolffberg (18) and the studies of Voit (19) substantiated to a large extent this principle. The theory that in diabetes sugar originates in part from amino products was strongly advocated by Müller (20); but the definite proof was first afforded by Stiles and Lusk (21) and by Berger (22). Furthermore, the studies of Neuberg and Langstein indicated that in normal rabbits the ingestion of alanine was followed by the appearance of lactic acid in the urine (23). Ringer and Lusk (24) and Dakin and Dudley (25) found that in the phlorhizinized animal alanine is completely converted into glucose. In brief, these various studies demonstrated that cleavage of proteins through the process of deamination, is followed by the conversion of part of the protein molecule to glucose.

Lusk (21) and his collaborators, as well as Falta, Grote and Staehelin (26), found an enhanced protein metabolism in dogs rendered diabetic whether with phlorhizin or by pancreatectomy. Okada and Hayashi found in the blood of depancreatized dogs a hyperaminoacidemia (27). Similar studies on diabetic patients indicated likewise an increase in the amino nitrogen content of the circulating blood (28, 29, 30). The latter, however, could not be substantiated by Greene and his co-workers (31).

The studies cited above indicate that in an inflamed area there is active proteolysis. Furthermore in the diabetic animal with increased protein catabolism glucose can readily originate from amino products. Therefore

it seems reasonable to assume that in foci of proteolysis such as exist in inflamed areas, the process of protein breakdown in a diabetic animal might possibly be considerably enhanced. It seems quite immaterial whether one abides by the non-utilization or the over-production theory of diabetes (32). In either case gluconeogenesis from proteins could occur at the site of inflammation, thus allowing for the gradual diffusion of sugar into the circulating blood. The following series of experiments indicates that this indeed seems to be the state of affairs.

Blood samples were withdrawn from the heart of normal dogs. Chemical studies were undertaken to determine the level of lactic acid (33), sugar (34), total protein (35), non-protein nitrogen (36), urea (37), amino acid nitrogen (38) and pH (39). The analytical method utilized in each case is indicated in the bibliographical reference. Pancreatectomy was then performed under nembutal anesthesia. The post-operative care, the method and duration of insulin administration have already been described. Studies on the blood chemistry were repeated several days later but prior to the injection of the irritant in the pleural cavity.

Samples of both exudate and blood were withdrawn at varying intervals and chemical determinations of nitrogenous and carbohydrate constituents were made on both types of material. At the end of the experiment the animal was usually sacrificed and a necropsy performed. Several animals received about 8 to 10 units of insulin throughout the duration of the inflammation in an endeavor to determine the influence of this substance on proteolytic activity in an acutely inflamed area. Control studies were also performed on several non-diabetic dogs having an acute pleural inflammation. The results of all experiments are listed in tables 2 and 3. The data are graphically shown in figures 5 and 6. The results of a type experiment to illustrate the progressive change in protein metabolism in the exudate of a diabetic compared to that of a non-diabetic dog are presented in figures 3 and 4.

The data can be conveniently summarized as follows: The lactic acid of diabetic exudates averages 89.29 mgm. per 100 cc. as compared with 58.75 mgm. in non-diabetic exudates. This represents an elevation of 52 per cent. The administration of insulin in depancreatized animals completely inhibits the rise in exudate lactic acid, the average level being brought down to 57.84 mgm. per cent (table 3, fig. 5). The average concentration of sugar in diabetic exudates is 452.4 mgm. per 100 cc. of the material. The level in exudates of non-depancreatized dogs averages 78.87 mgm. There is thus an average increase of 473.6 per cent in the sugar content of diabetic exudates. The level is distinctly reduced with repeated insulin administration, the average figure being 191.7 mgm. of sugar per 100 cc.

The observations regarding the status of protein metabolism in exudates

TABLE 2

Studies on carbohydrate and nitrogenous metabolism in exudates of diabetic and non-diabetic dogs

DOG NUMBER		DURATION OF INFLAMMATION		CARBOHYDRATE METABOLISM				NITROGENOUS METABOLISM							
				Diabetic exudate		Non-diabetic exudate		Diabetic exudate				Non-diabetic exudate			
Diabetic	Non-diabetic	Diabetic dogs	Non-diabetic dogs	Lactic acid	Sugar	Lactic acid	Sugar	Total protein	NPN	Urea	Amino acid N	Total protein	NPN	Urea	Amino acid N
		hrs.: mins.	*hrs.: mins.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*gm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*gm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
1*	17†	7:05	6:15	89.1	422.9	119.3	44.1	3.7	74.1	72.0	9.7	4.4	38.0	29.0	8.9
3	11	15:45	23:00	167.3	422.8	40.6	73.8	4.1	84.7	127.0	12.1	3.5	40.2		6.9
18	12	16:35	23:30	47.0	419.8	30.2	86.2	3.6	69.1		12.0	4.8	49.0		5.5
4§	14	24:30	24:00	44.1	344.8	52.0	63.7	3.6	49.7		11.2	4.9	42.4		6.5
7	20	33:12	24:25	72.8	427.5	61.9	91.2	4.2	62.5	42.0	18.6	4.9	36.9		9.6
19	15	36:00	25:30	91.6	153.4	76.2	71.3	4.4	98.0		10.9	4.3	44.8		7.3
8	13	38:00	34:15	92.6	869.8	57.9	50.9	3.8	100.0		11.8	4.3	69.0		7.5
6	16	25:08	42:15	132.2	629.2	28.7	124.0	4.0	72.2	60.5	12.2	4.5	32.0	26.0	5.4
6	9	48:10	24:45	66.8	381.7‡	25.7	131.1	4.3	115.4	89.0	17.4	4.5	31.6	40.5	6.7
	9		47:50			95.0	52.4					5.3	41.7	42.5	9.6
Average				89.20	452.4	58.75	78.87	3.97	80.63	78.1	12.88	4.54	42.50	34.5	7.39

* Succumbed 25 min. after thoracentesis.

† Splenectomized several days preceding the injection of the irritant. Died 40 min. after thoracentesis.

‡ Fall in sugar may in part be referable to starvation.

§ Sample of exudate withdrawn from left pleural cavity, although irritant originally introduced into right thoracic cavity.

TABLE 3

Effect of inflammation on some of the carbohydrate and nitrogenous constituents of exudates in depancreatized dogs treated with low doses of insulin

DOG NUMBER	DURATION OF INFLAMMATION	LACTIC ACID	SUGAR	TOTAL PROTEIN	NPN	UREA	AMINO ACID NITROGEN
	hrs.:mins.	*mgm./100 cc.*	*mgm./100 cc.*	*gm./100 cc.*	*mgm./100 cc.*	*gm./100 cc.*	*mgm/100 cc.*
21	6:05	68.3	40.7*	5.9	21.3	14.0	3.9
22	6:20	43.6	55.7*	4.6	13.8	16.0	3.9
	27:50	21.8	251.0	4.0	47.2	34.5	12.7
23	7:23	71.3	303.1	4.0	22.0		6.0
	25:00	43.9	219.8	3.9	27.6		9.5
	47:10	31.7	383.1	3.6	40.2		10.6
19	11:30	124.3	88.5	5.4	54.0		9.4
Average		57.84	191.7	4.49	32.3	21.5	8.0

* Insulin administered only a few hours prior to removal of sample of exudate.

are of even greater interest. The average total proteins per 100 cc. of exudate in diabetic dogs is 12.56 per cent lower than that encountered in normal animals. The actual figures are 3.97 and 4.54 grams respectively

(table 2). In the exudates of depancreatized and insulin-treated dogs the average total protein concentration of 4.49 approximates that found in the non-diabetic (table 3). The products of protein catabolism show much more striking differences in the three groups of animals. The non-protein nitrogen of exudates in diabetic dogs averages 80.63 mgm. per 100 cc. of material. This is in sharp contrast to the average concentration of 42.56 mgm.

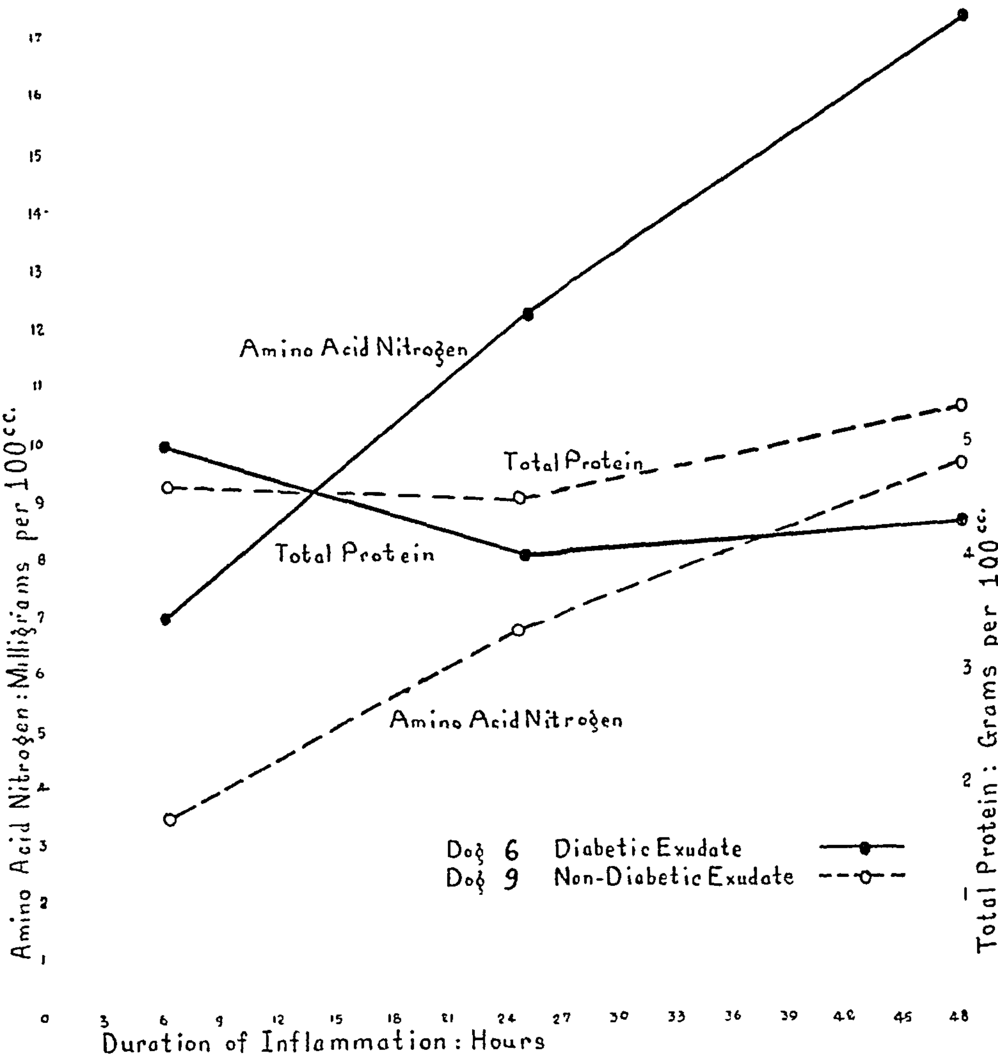

Fig. 3. Enhanced proteolysis in inflamed area of diabetic dog. Effect of experimental diabetes on amino acid nitrogen and total protein of exudates.

in the control non-diabetic preparations. A comparison of these figures indicates an increase of 89.45 per cent in the NPN of exudates of experimental animals. The urea in exudates of diabetic dogs averages 78.1 mgm. per 100 cc., whereas the exudates of control dogs yield an amount averaging 34.5 mgm. per cent. This represents a difference of 126.3 per cent. Finally the amino acid nitrogen concentration of diabetic exudates averages 12.88 mgm. per cent. The average amino acid nitrogen in con-

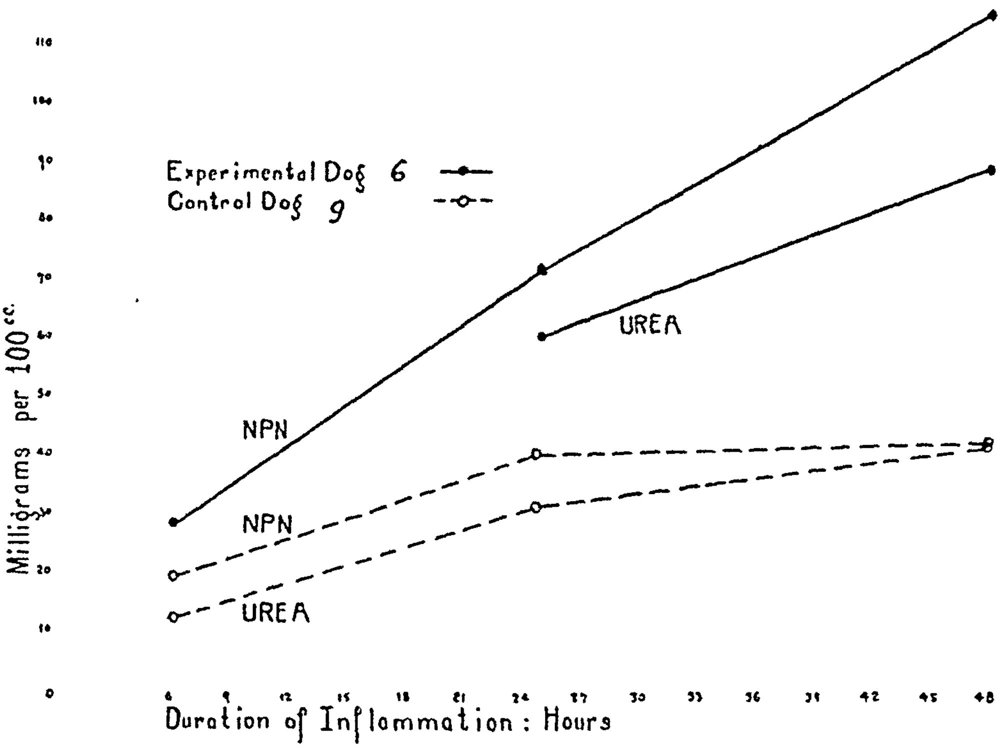

Fig. 4. Effect of experimental diabetes on non-protein nitrogen and urea concentrations of exudates.

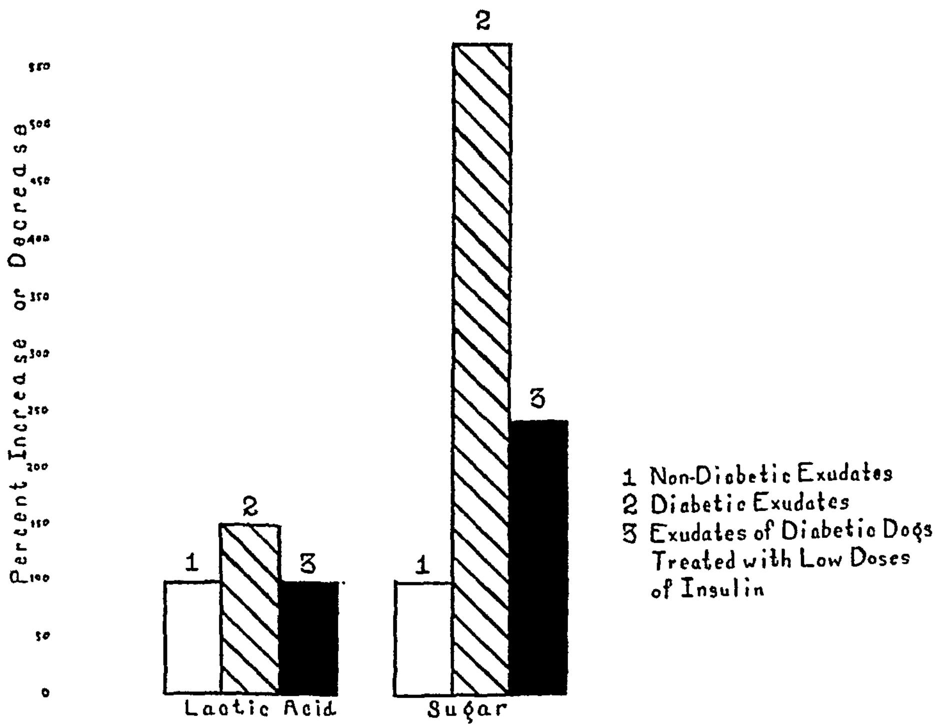

Fig. 5. Effect of inflammation on the carbohydrate constituents of exudates in diabetic and non-diabetic dogs.

trol exudates is found to be only 7.39 mgm. The average increase in the amino acid nitrogen of exudates in diabetic dogs is therefore 74.29 per cent. In brief, these figures on non-protein nitrogen, urea and amino acid nitrogen of exudates reveal a markedly enhanced degree of proteolysis at the site of inflammation of depancreatized dogs (table 2).

It is well known that insulin inhibits the formation of glucose from non-carbohydrate precursors (40, 9).

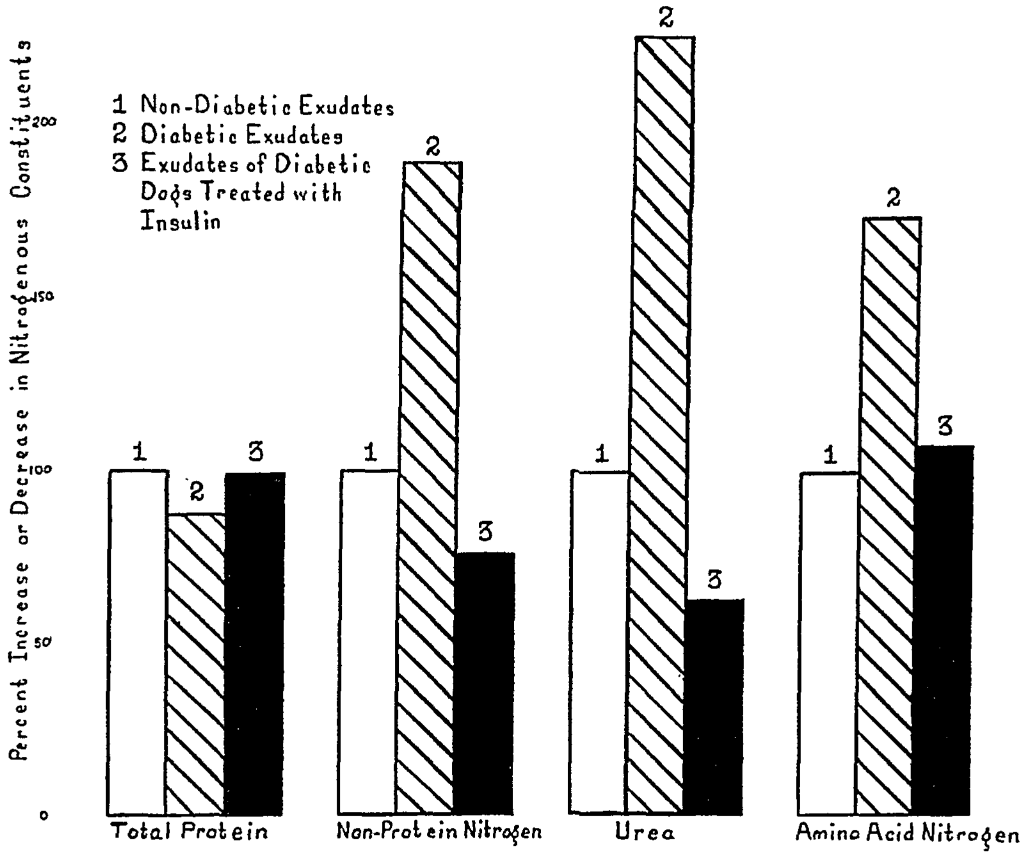

Fig. 6. Effect of inflammation on the nitrogenous constituents of exudates in diabetic and non-diabetic dogs.

There is evidence that the administration of insulin tends to lower the amino acid nitrogen of the blood. Luck and his associates showed that subconvulsive doses of insulin lower the amino acid content of the blood of dogs, rabbits, rats and man (41, 42). They were led to the conclusion that under the conditions of their experiments insulin seems both to increase the rate of amino acid catabolism and at the same time to inhibit the process of protein hydrolysis by which amino acids are generated. These observations on dogs were confirmed by Kerr and Krikorian (43); but Bischoff and Long were unable to substantiate the findings on rabbits (44). The studies were again followed up at a later date by Powers and

Reis (45). They confirmed the above-mentioned observations on rabbits reported by Luck and his collaborators; but they were unable to corroborate the additional claim that insulin tends to induce a rise in urea nitrogen. Farr and Alpert arrived at essentially the same conclusion (46). Subcutaneous injections of insulin produced sharp decreases in plasma amino acids with no significant changes in blood urea.

Bach and Holmes (47) reported that insulin decreased *in vitro* glucose formation by excised liver tissue. This reduction was accompanied by a diminution in urea formation. Stadie and his associates confirmed the inhibition of oxidative deamination by insulin on d-amino acids (48). Mirsky studied the influence of insulin on the protein metabolism of dogs (49). His data suggested that insulin exerts a nitrogen-sparing action by decreasing the rate of oxidative deamination in the liver, while at the same time it enhances the rate of amino acid utilization by muscles for protein synthesis. This investigator pointed out that "In experimental or clinical diabetes mellitus, insulin produces a profound decrease in urinary nitrogen excretion which is concomitant with an increase in carbohydrate retention."

The present series of observations indicates that pancreatectomized dogs with a superimposed pleurisy manifest not only a sharp rise in blood and exudate sugar but also a pronounced degree of proteolysis at the site of inflammation. If the excess sugar formation in the inflamed area is primarily derived from the local breakdown of proteins, the administration of insulin should both depress the level of glucose and inhibit the enhanced protein catabolism. Studies have therefore been undertaken in depancreatized dogs receiving twice daily about 8 to 10 units of insulin throughout the duration of a pleural inflammation induced as described previously.

The results of the experiments are shown in table 3. A convenient graphical comparison of the observations with the data obtained in non-diabetic and in diabetic dogs receiving no insulin during the period of the experiment is also shown in both figures 5 and 6. The results may be briefly summarized as follows: The average lactic acid of exudates in insulin-treated depancreatized dogs is essentially the same as found in non-diabetic dogs; the actual figures are 57.84 mgm. and 58.75 mgm. respectively. This indicates a significant decline in the lactic acid content when compared with the level in exudates of untreated diabetic animals. The average sugar of exudates likewise shows a marked drop in concentration; the content in the exudate of insulin-treated dogs being 191.7 mgm., as against 452.4 mgm. in untreated animals. When compared with the glucose level of exudate in control animals, the results represent an increase in sugar of 143.1 per cent in the insulin-treated group, and an increase of 473.6 per cent in the untreated diabetic dogs. It is to be noted

that in individual instances when insulin was administered a relatively short interval prior to removal of the exudate sample, the level of sugar was invariably very low (dogs 21 and 22, table 3).

The repression by insulin of glucose and lactic acid formation is likewise reflected in the protein catabolism. As already mentioned the average total protein concentration of exudate in the insulin-treated group is higher than that found in the untreated diabetic animals. It is essentially at the same level as encountered in control animals. The non-protein nitrogen and the urea of exudates are both considerably reduced by repeated insulin administration (table 3, fig. 6). The average contents are 32.3 mgm. and 21.5 mgm. respectively. This represents a decrease of 24.1 and 37.7 per cent when compared with the levels of these same constituents in exudates of control animals (table 3, fig. 6). This is in contrast to the marked elevation of the nitrogenous products in exudates of depancreatized dogs receiving no insulin (table 2). Finally, the average amino acid nitrogen of exudates after insulin administration is only increased 8.3 per cent in contrast to the significant rise of 74.3 per cent in exudates of untreated diabetic dogs.

In brief, the data show without any doubt that the enhanced proteolysis at the site of inflammation of diabetic dogs is completely held in abeyance by the administration of insulin. Since this fact is also paralleled by a corresponding appreciable reduction in glucose concentration, it is reasonable to infer, in view of what is known concerning the formation of sugar from proteins in diabetic animals, that the most likely source of gluconeogenesis at the site of inflammation is primarily protein in nature.

There is a further, though perhaps indirect, type of evidence in support of the view that the excess sugar and lactic acid formation in exudates is, at least in large part, probably derived from proteins. In earlier studies the writer has shown that the local acidosis which develops in an inflamed area is referable to glycolytic processes. The conversion of sugar to lactic acid was found to be the primary factor responsible for the gradual increase in hydrogen ion concentration at the site of an acute inflammation (13, 14, 52). The observations revealed a reciprocal type of relationship between the concentration in exudate of sugar and lactic acid. But in the present series of experiments on diabetic exudates, no such consistent relationship is detectable. The characteristic picture encountered is illustrated in figure 7. The reciprocal relationship between sugar and lactic acid, clearly manifest in the exudate of a non-diabetic dog (no. 9), is absent in the material withdrawn from the pleural cavity of a depancreatized dog (no. 6). This seems to indicate that in all probability the level of lactic acid in the exudate of a diabetic animal is not wholly conditioned by the sugar concentration. The graph clearly shows a parallelism between the level of these two substances rather than the usual type of

reciprocal relationship found in the exudate of a non-diabetic dog. It is therefore quite likely that this state of affairs is referable to an appreciable amount of sugar and lactic acid arising directly from protein breakdown.[3]

Carbohydrate and protein metabolism in the circulating blood of depancreatized dogs with a superimposed acute inflammation. The foregoing series of observations indicates that in a depancreatized dog a focus of inflammation manifests not only a disturbance in carbohydrate metabolism but also a concomitant and significant rise in protein catabolism.

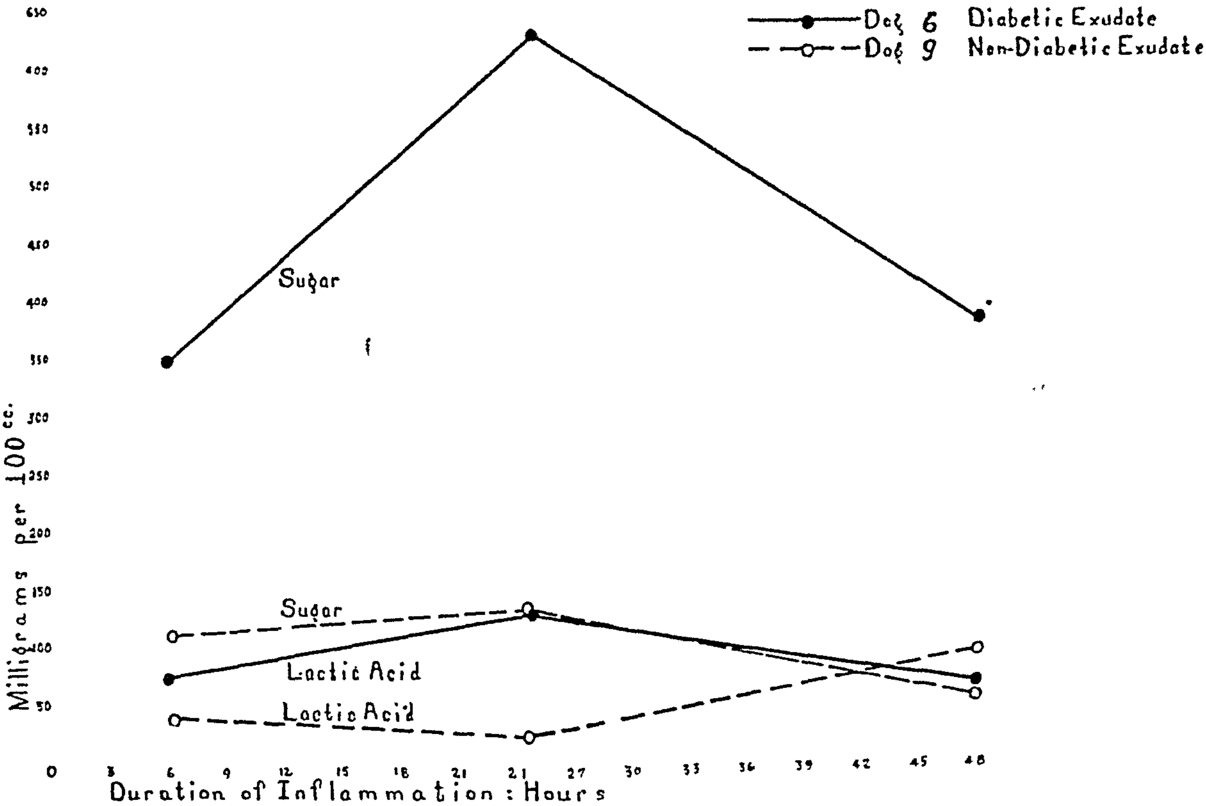

Fig. 7. Absence of reciprocal relationship between sugar and lactic acid in the exudate of a diabetic dog.

How do these changes affect the levels of the nitrogenous and carbohydrate constituents of the blood?

In the first place, is the high exudate sugar referable to a diffusion of this substance from the circulation? Two sets of evidences would tend to rule out such a conclusion. As pointed out in an earlier section, the

[3] Does any of the formed sugar in the inflamed area arise from fatty acids? This remains a possibility, but it is to be borne in mind that the whole question of gluconeogenesis from fatty acids is still in a controversial state (32, 50). Preliminary observations by the method of Behre and Benedict (51) indicate that a diabetic dog with inflammation fails to show in its exudate a significantly higher level of total acetone bodies than is found in the exudate of a non-diabetic dog.

hyperglycemia is intensified only when there is a concomitant inflammation. Pancreatectomy *per se* fails to induce the excessively high blood sugar level which rapidly develops with an accompanying inflammation. Furthermore, a comparative study of exudate and blood sugar in diabetic animals indicates that the exudate sugar tends to be at a higher level than

TABLE 4

A comparison of the exudate and blood sugar concentration in diabetic and non-diabetic dogs

DOG NUMBER	APPROXIMATE DURATION OF INFLAMMATION	BLOOD SUGAR	EXUDATE SUGAR
Diabetic animals			
	hrs. : mins.	*mgm./100 cc.*	*mgm./100 cc.*
24	7:40	454.5	579.7
25	11:35	241.0	368.3
18	16:35	295.7	419.8
18	41:00	310.7	442.8
8	13:40	482.0	470.7
8	38:00	652.5	869.8
8	63:00	655.7	761.9
6	6:15	311.5	349.9
6	25:15	630.9	629.2
7	33:30	442.9	427.5
Average		447.7	532.0
Non-diabetic animals			
11	23:00	74.8	73.8
14	24:00	64.2	63.7
12	23:30	55.6	86.2
15	25:30	70.8	71.3
20	6:45	83.9	85.3
20	24:25	111.1	91.2
20	78:40	110.7	46.7
13	10:30	83.8	25.4
13	34:15	61.0	50.9
13	57:35	95.0	40.5
Average		81.1	63.5

that of blood. The results of such observations are compiled in table 4. Animals that received considerable insulin or else which were moribund or at least extremely ill at the time of withdrawal of blood and exudate samples were not included in this series. Studies were also extended to similar specimens from non-diabetic dogs (table 4). The results indicate an average blood sugar in diabetic animals of 447.7 mgm. per 100 cc.

whereas the exudate sugar concentration in these same animals was found to average 532.0 mgm. per cent. By contrast similar comparative studies in a series of 10 control non-diabetic specimens indicate an average blood sugar of 81.1 mgm. per cent, and an average exudate sugar of 63.5 mgm. per cent. These facts show that the exudate sugar of diabetic animals tends to be higher than the corresponding blood sugar, while the reverse seems to exist in non-diabetic dogs. This state of affairs is not wholly surprising inasmuch as the available evidence, described previously, indicates that the excess exudate sugar of diabetic animals is at least in large part derived at the site of inflammation from enhanced local protein breakdown which in turn is readily held in abeyance by insulin administration. As pointed out above, in non-diabetic animals the exudate sugar level tends often to be lower than the corresponding blood sugar. This is most likely referable, as shown by the writer in earlier studies (13), to the higher degree of glycolysis at the site of inflammation than in the blood stream.[4]

It is of significance to point out that the elevated levels of both carbohydrate and protein metabolic products in exudates of diabetic animals are likewise reflected in the circulating blood. The evidence indicates that the heightened concentration of these substances in the blood is evidently referable to absorption from the inflamed area. This in no way contradicts the earlier findings of the writer on the fixation at the site of an acutely inflamed area of various materials (53, 54, 14). The degree of fixation is referable to the intensity of local injury and also to the size of the particle (55, 14). For instance, the writer has demonstrated that proteins are less readily retained than bacteria or graphite particles (56, 57). Miller has essentially substantiated this concept by demonstrating that diffusible substances are rapidly absorbed from an inflamed area (58).

The results of these studies are shown in table 5 and on figure 8. Determinations on blood samples were made before pancreatectomy (column B, table 5), several days after the operation (column A), and when there was an accompanying pleural inflammation (column W.I.). The

[4] There are doubtless several factors which regulate the concentration and distribution of diffusible substances between exudate and blood. Some of these may be briefly enumerated as follows: *a*, diffusibility which tends to equalize the concentration of the materials in both types of fluids; *b*, difference in rate of glycolysis between exudate and blood which thus favors the more rapid degradation of the sugar molecule in exudates (13); *c*, the early appearance of impaired local circulation in an acutely inflamed area in the form of lymphatic blockade (14) might conceivably play a rôle in the speed of equilibration of even diffusible substances between blood and exudate. Notwithstanding these various factors, as well as others, it is interesting to note that the enhanced degree of local gluconeogenesis from proteins in the inflamed area of a diabetic animal seems to transcend the effect of these factors, so that the exudate sugar concentration still tends to be higher than that found in the blood (table 4).

data indicate quite clearly that pancreatectomy *per se*, except for the rise in blood sugar, fails to induce any other very significant changes. On the other hand with the development of a severe inflammatory reaction in the pleural cavity there is not only an enhancement in the blood sugar and lactic acid, but there is also a marked rise in the blood non-protein nitrogen and urea. The amino acid nitrogen likewise shows an appreciable increase in concentration; while the total proteins, with a concomitant pleural inflammation, reveal a slight drop. With pancreatectomy, the concentration of these various carbohydrate and nitrogenous constituents fail to rise in the systemic circulation to the high levels reached when

TABLE 5

Effect of pancreatectomy and of inflammation on some of the carbohydrate and nitrogenous constituents in the circulating blood

DOG NO.	DURATION OF INFLAMMATION	LACTIC ACID mgm./100 cc.			SUGAR mgm./100 cc.			TOTAL PROTEIN gm./100 cc.			NPN mgm./100 cc.			UREA mgm./100 cc.			AMINO ACID N mgm./100 cc.		
		B	A	W.I.	B	A	W.I.	B	A	W.I.	B	A	W.I.	B	A	W.I.	B	A	W.I.
	hrs.: mins.																		
1*	7:20	24.8	10.6	30.7	109.1	311.7	497.3	5.9	3.6	4.9	51.9	41.5	86.5	63.0	21.5	53.0	8.3	18.9	8.9
3	15:45	12.9	32.2	71.5	104.2	257.1	522.2	5.1	4.9	4.0	34.3	35.9	83.3	33.5	31.0	124.5	6.9	6.6	10.2
4	24:30	18.8	17.3	12.9	90.1	240.9	412.4	5.4	4.8	4.5	43.0	37.2	49.0				7.4	10.6	8.6
7	33:32	9.9	19.3		104.6	335.3	442.9	4.9	5.7	4.9	26.2	35.1	63.5	18.0	23.0	41.0	7.9	8.0	14.9
19	36:00	19.3	18.4	20.3	98.3	222.2	202.2	5.9	5.5	4.5	32.1	32.0	112.0				4.8	7.4	8.5
8	38:00	16.3	36.1	33.7	73.6	234.0	652.5†	5.9	5.9	5.4	53.6	31.5	106.7				6.8	6.8	9.8
6	25:18	11.1	10.4	23.8	98.2	257.4	630.9	6.6	4.9	3.9	34.0	25.8	70.3	36.5	11.5	51.0	6.0	8.5	10.4
6	48:15			23.8			430.1‡			4.1			109.8			81.5			13.0
Average..		16.16	20.61	30.96	96.87	265.51	473.81	5.67	5.04	4.53	39.3	34.14	85.14	37.75	21.75	70.2	6.87	9.54	10.54

B = Determinations of blood samples withdrawn before pancreatectomy.
A = Determinations of blood samples withdrawn several days following pancreatectomy.
W.I. = Determinations of blood samples withdrawn when there is a concomitant pleural inflammation of varying duration.
* Succumbed 10 min. after removal of blood sample.
† Blood sugar when inflammation was about 44 hours' duration.
‡ Fall in sugar may be referable to starvation.

there is an additional superimposed pleurisy. This would suggest that their elevation is referable to a gradual diffusion or absorption from the site of inflammation.

Similar studies were repeated in a number of non-diabetic animals having an acute inflammation induced likewise by an intrapleural injection of turpentine. The results are shown in table 6 and figure 9. It is clear that neither the carbohydrate nor the nitrogenous metabolism is essentially altered.

The above observations therefore warrant the conclusion that the marked rise in protein catabolism occurring at the site of an acutely in-

flamed area of a depancreatized dog is likewise reflected in the circulating blood. Furthermore, the data indicate that the enhanced proteolysis in inflamed areas of diabetic animals offers a reasonable explanation for local gluconeogenesis from proteins. The surplus glucose formed, in turn, dif-

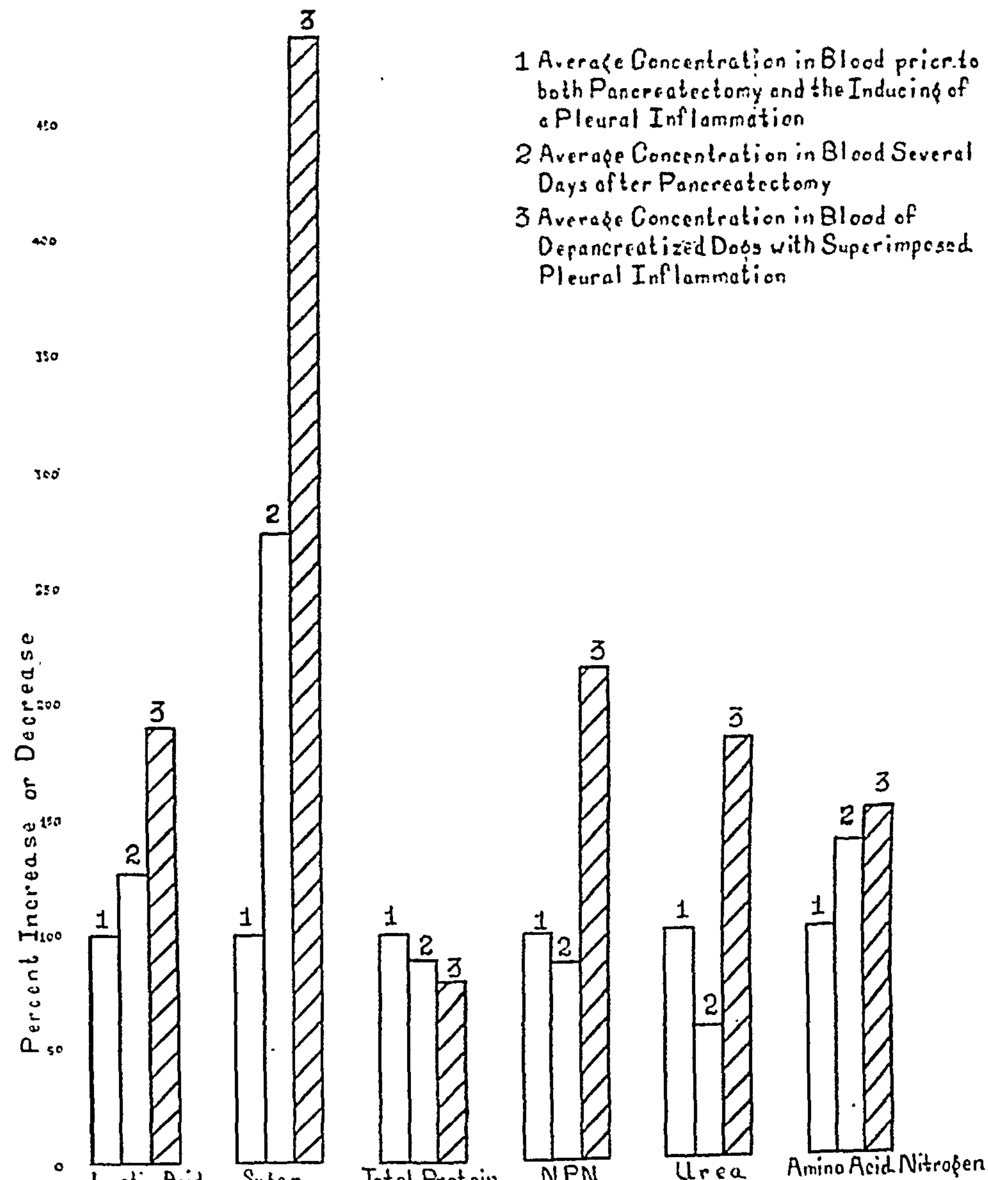

Fig. 8. Effect of inflammation on the concentration of some of the carbohydrate and nitrogenous constituents in the blood of diabetic dogs.

fuses from the area of inflammation into the blood stream thus giving rise to an enhanced state of hyperglycemia.

The increased proteolysis found in diabetic exudates has been shown to be inhibited by repeated administration of insulin (table 3, fig. 6).

In a like manner the parallel increase of these constituents in the circulating blood of a depancreatized dog with an induced pleurisy can readily be repressed by insulin treatment. The results of such observations appear in table 7. The average blood sugar is found to have dropped to an even lower level than that seen prior to the introduction of the inflammatory irritant. It is therefore not surprising to find the NPN and amino acid nitrogen concentration of the blood relatively normal (table 7). These

TABLE 6

Effect of inflammation on some of the carbohydrate and nitrogenous constituents in the circulating blood of non-diabetic dogs

DOG NUMBER	DURATION OF INFLAMMATION	LACTIC ACID		SUGAR		TOTAL PROTEIN		NPN		UREA		AMINO ACID N	
		mgm./100 cc.		mgm./100 cc.		gm./100 cc.		mgm./100 cc.		mgm./100 cc.		mgm./100 cc.	
		B	W.I.	B	W.I.	B	W.I.	B.	W.I.	B	W.I.	B	W.I.
	hr.:mins.												
9	6:25	18.8	17.8	120.3	95.9	6.8	6.2	27.8	13.2	?	15.5	7.2	3.4
	24:50		?		118.1		5.6		35.1		25.0		6.1
	48:05		11.9		109.0		5.8		34.5		35.0		5.6
20	7:00	19.8	19.8	81.7	83.9	6.0	5.4	50.9	33.2			7.0	6.9
	24:45		12.4		111.1		5.2		32.1				7.5
13	10:40	14.9	14.4	112.9	83.8	6.0	5.9	31.4	34.3			7.1	6.0
	34:25		16.3		61.0		5.9		68.4				5.8
	57:45		10.9		95.0		5.8		34.5				5.7
11	23:00	10.9	8.4	71.4	74.8	5.3	4.5	27.8	33.8			5.1	5.1
12	23:10	14.4	10.9	69.8	55.6	6.5	5.9	?	51.0			8.3	5.4
14	24:50	10.9	10.9	63.7	64.2	6.7	5.5	37.2	33.2			4.4	5.2
15	25:05	9.4	8.9	69.3	70.8	7.0	5.4	36.0	44.2			6.8	5.7
26*	6:00		52.5		133.8		4.6		24.1		20.0		6.2
	22:00		22.5		103.0		4.8		22.1		14.0		7.3
Average.....		14.16	16.74	84.16	90.0	6.33	5.46	35.18	35.26		21.9	6.56	5.85

B = Determinations in blood samples withdrawn prior to inducing inflammatory reaction.

W.I. = Determinations in blood samples withdrawn when there was an inflammation of varying duration.

* Partial pancreatectomy prior to inducing the inflammatory reaction.

findings are doubtless referable to a repression by insulin of proteolysis at the site of inflammation. It is interesting to note in this connection that Geelmuyden, recently reviewing the function of insulin, called attention to the fact that "Its most important action is to inhibit the formation of carbohydrate from non-carbohydrates" (59).

The cytological picture of inflammatory exudates in diabetic dogs. With the attending enhanced degree of proteolysis in an area of inflammation

in a diabetic dog, it is conceivable that the cells might manifest detectable signs of protoplasmic injury.

The writer demonstrated several years ago that the cytological picture in an inflamed area is conditioned by the hydrogen ion concentration (52). Polymorphonuclear leukocytes survive a pH above 7.0. At a pH of 6.9

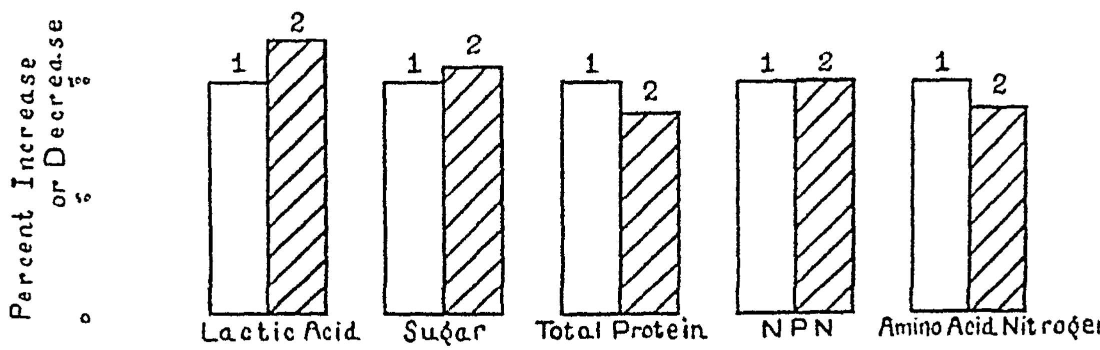

Fig. 9. Effect of inflammation on some of the carbohydrate and nitrogenous constituents in the blood of non-diabetic dogs. 1, average concentration in blood prior to the inducing of a pleural inflammation. 2, average concentration in blood concomitant with an acute pleural inflammation.

TABLE 7

Effect of inflammation on some carbohydrate and nitrogenous constituents of the blood in depancreatized dogs treated with low doses of insulin

DOG NUMBER	DURATION OF INFLAMMATION	BLOOD SUGAR PRIOR TO THE INTRODUCTION OF THE IRRITANT	BLOOD SUGAR SUBSEQUENT TO THE INTRODUCTION OF THE IRRITANT	NPN PRIOR TO THE INTRODUCTION OF THE IRRITANT	NPN SUBSEQUENT TO THE INJECTION OF THE IRRITANT	AMINO ACID NITROGEN PRIOR TO THE INTRODUCTION OF THE IRRITANT	AMINO ACID NITROGEN SUBSEQUENT TO THE INJECTION OF OF THE IRRITANT
	hrs.:mins.	*mgm./100 cc.*	*mgm./100 cc.*	*mgm./100 cc.*	*mgm./100 cc.*	*mgm./100 cc.*	*mgm./100 cc.*
21	6:10	184.2	44.6*	20.6	21.5	4.0	3.7
22	6:35	156.0	71.5*	27.5	14.2	8.6	4.3
	28:00		241.0		37.2		10.7
	47:25		168.1		35.2		12.2
23	7:25	282.6	281.8	?	20.5	?	5.5
	25:05		242.7		29.1		9.2
	47:10		235.3		41.0		10.6
19	11:30	222.2	166.7	32.0	42.0	7.4	5.8
Average.........		211.25	181.46	26.7	30.09	6.67	7.75

* Insulin administered several hours prior to withdrawal of blood sample.

or 6.8 these cells exhibit marked signs of injury and degeneration; but the macrophages appear relatively normal. At a lower pH of 6.5 or thereabouts all forms of leukocytes are injured and frank pus is in evidence. *In vitro* observations further support the view that the cytological picture in an inflamed area is a function of the pH (60). The mechanism of local acidosis was found to be primarily referable to a glycolytic process (13).

The formation of lactic acid from sugar breakdown seems to be mainly responsible for the observed changes in pH and the accompanying cytological shift from polymorphonuclear to mononuclear phagocytes in an inflamed area.

TABLE 8

Effect of inflammation on the pH and the cytological picture of exudates in depancreatized and non-depancreatized animals

DOG NUMBER	DURATION OF INFLAMMATION	pH		CYTOLOGY OF EXUDATE		APPEARANCE OF CELLS IN EXUDATE
		Blood	Exudate	Per cent of polymorphonuclears	Per cent of mononuclear phagocytes	
			Experimental depancreatized group			
	hrs.:mins.					
1	7:05	7.42	7.1	Very few cells per field		Swollen, degenerated
3	15:45	7.47*	7.0*	22	78	Degenerated
18	16:35	7.4	7.3	92	8	Normal appearance
4	24:30	7.6	6.95	47	53	Conspicuous vacuolization
6	25:08	7.55	6.9	2	98	Extensive vacuolization; degenerated cells
7	33:12	7.55	7.1	76	24	Swollen and vacuolated
19	36:00	7.55	7.03	88	12	Fairly normal
8	38:00	7.70	7.0	Cells beyond recognition; degenerated, swollen and vacuolated		
			Control, non-depancreatized group			
11	23:00	7.47	7.2	90	10	Normal
12	23:30	7.4	7.33	92	8	Normal
14	24:00	7.4	7.28	88	12	Normal
15	25:30	7.38	7.05	82	18	Normal
20	24:25	7:55	7.13	74	26	Normal
13	34:15	7.43	7.07	76	24	Polymorphs are swollen, distorted, vacuolated
16	42:15	7.55	7.4	78	22	Normal
9	24:45	7.57	7.5	82	18	Normal
9	47:50	7.63	7.03	66	34	Polymorphs are swollen, vacuolated and degenerated

* pH measured at 36 to 38°C.; all others at room temperature.

Studies on the cytological picture and the hydrogen ion concentration of exudates in diabetic and non-diabetic dogs were accordingly undertaken. The method utilized to measure the pH has been previously described in detail (52, 13). Cellular smears of exudates were all stained by the usual Wright method.

The results of all observations are summarized in table 8. It is evident

that the pH of exudates in diabetic dogs tends to be somewhat lower than that found in exudates of non-diabetic animals. The differences in pH are fairly well correlated with the respective concentrations of lactic acid in the exudates of both groups of animals (cf. tables 2 and 8).

The cytological picture reveals conspicuous differences. The cells from a diabetic exudate are usually degenerated, swollen or vacuolated. Some cells are unidentifiable owing to the extent of injury. On the other hand, the cells from an exudate of a non-diabetic dog are, as a rule, perfectly normal unless the inflammation is of long duration (i.e., about 2 days). The exudates in non-diabetic animals exhibit in the first twenty-four hours of inflammation a predominance of polymorphonuclear leukocytes. This is in conformation with previous findings (52). On the other hand, exudates from depancreatized animals do not show in the same interval a consistent predominance of polymorphonuclear cells. Histological studies of the pleura in both groups of animals corroborate further the cytological picture of exudates.

The above observations indicate that the enhanced proteolysis in an acutely inflamed area of a diabetic dog not only offers an explanation for active gluconeogenesis, but it also accounts for the increased degree of local tissue damage encountered in diabetes complicated by inflammation.

Discussion. The present results offer a reasonable explanation for the enhanced diabetes in depancreatized dogs with an accompanying acute inflammation. The mechanism, in brief, seems to be primarily referable to an augmented degree of proteolysis at the site of inflammation. The increased protein catabolism seems responsible for the marked degree of local gluconeogenesis. The surplus glucose derived from the degradation of the protein molecule in the injured area gradually diffuses into the systemic circulation, enhancing thus further the elevated blood sugar level.

The findings and the inferences drawn raise several questions. What controlling process in the diabetic organism actually enhances local proteolysis at the site of inflammation? Is gluconeogenesis from proteins in a diabetic animal restricted only to inflammatory foci and to the liver? May it not be true of any area where there is tissue damage, such as, for instance, an arteriosclerotic lesion? To what extent does diabetes interfere with normal protein synthesis, and if so, what possible effects may this have on various immunological phenomena such as antibody formation?

Long (11) recently discussed the possibility that protein catabolism leading to gluconeogenesis may be referable to activity of the anterior pituitary gland. Moreover, the participation of the adrenals, possibly the thyroid, and the liver in carbohydrate and protein metabolic distur-

bances is also not to be overlooked. One of the functions of the adrenal cortex is presumably to regulate, at least to some extent, the level in the circulation of various electrolytes, particularly sodium and potassium (61). It is of interest in this connection that several determinations on the sodium and potassium concentration of exudate and blood of diabetic and non-diabetic dogs have revealed no appreciably significant difference.

As pointed out at the beginning of this paper, in spite of the many obvious similarities, there are some differences between human diabetes and experimental diabetes induced by pancreatectomy. It is therefore of definite importance to extend the present studies to human diabetic patients whose disease is complicated by marked inflammatory processes. It is conceivable that a diabetic individual with only minimal infection may not reveal in the blood stream the striking proteolytic changes detected in dogs having extensive pleural involvement.

CONCLUSIONS

An acute pleural inflammation in a dog fails to alter its blood sugar. On the other hand, the presence of an acute inflammatory reaction in a dog, rendered diabetic by pancreatectomy, induces a rapid and marked elevation in the blood sugar level.

The extent of local proteolysis at the site of an acute inflammation in a diabetic dog is considerably more pronounced than that found in a non-diabetic animal. This is indicated by a lower concentration of the total proteins and a correspondingly higher concentration of urea, non-protein nitrogen and amino acid nitrogen than is encountered in the exudative material of a non-diabetic animal.

The enhanced protein catabolism in the inflamed area of a diabetic animal is correlated with a marked elevation in both exudate sugar and exudate lactic acid. Insulin administration reduces not only the level of sugar and lactic acid in such diabetic exudates; but this substance diminishes as well the degree of local proteolysis. This fact supports the view that gluconeogenesis at the site of an acute inflammation in a diabetic animal originates from proteins through deamination of the molecule.

Enhanced local proteolysis in the inflamed area of a diabetic animal implies increased tissue damage. The cytological picture of a diabetic exudate indicates that polymorphonuclear leukocytes manifest pronounced signs of cellular injury when compared with similar cells derived from a normal exudate.

The biochemical changes encountered in the exudate of a depancreatized dog are similarly reflected in its circulating blood. Besides an elevation in blood sugar level, there is also an increase in the blood concentration of non-protein nitrogen, urea and amino acid nitrogen. Studies after pan-

createctomy or splenectomy, and a comparison of the relative glucose concentrations in exudate and blood, indicate that the raised levels in carbohydrate and nitrogenous constituents of the systemic circulation are neither due to the diabetes *per se* nor to the operative procedure. The elevation in the levels of these substances is referable to an absorption from the area of acute inflammation which complicates the diabetic disorder. Insulin by inhibiting glucose formation in the inflamed area likewise prevents a rise in the blood stream of intermediary products of carbohydrate and protein metabolism.

The available evidences, therefore, support the view that the mechanism of enhanced diabetes with concomitant inflammation might well be referable primarily to an increased local proteolysis in the inflamed area, favoring a combined picture of increased tissue damage with a corresponding elevation in glucose formation; the glucose, in turn, gradually diffuses into the systemic circulation.

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PHOSPHOLIPIDS AS A SOURCE OF ENERGY FOR MOTILITY OF BULL SPERMATOZOA[1]

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In a recent publication (2) it was shown that bull spermatozoa in sugar-free medium maintained motility only in the presence of air. Since deprivation of oxygen is not harmful to spermatozoa in the presence of glucose, we concluded that the spermatozoa were utilizing their intracellular reserves by an oxidative process. The intracellular substance utilized in the oxidative process appeared to be phospholipid. Since the respiration of bull spermatozoa was greatly *decreased* by adding glucose to the suspension medium, the spermatozoa apparently obtain energy preferentially from glycolysis and call upon the oxidative processes *as a source of energy* only when deprived of glycolyzable sugars (2).

While the above-mentioned work was in progress we were also studying the egg yolk-buffer medium (3, 4) in an attempt to determine which constituents of egg yolk were responsible for the effectiveness of this medium in promoting and maintaining an excellent degree of motility for long periods of time. Fractionation studies showed that the protein and neutral fat portions of egg yolk had no beneficial effect in preserving motility of spermatozoa in buffered media. In fact, the neutral fat rendered the spermatozoa immotile. The phospholipid fraction of the egg yolk, however, greatly prolonged the motility of spermatozoa in a buffered medium.

This report deals with experiments designed to study the manner in which the added phospholipids exert their effect on motility. Various preparations of phospholipid were made and their effect on the chemical and physiological behavior of spermatozoa studied.

EXPERIMENTAL. The semen used in the work was obtained from healthy dairy bulls by means of an artificial vagina. The method for motility observations and lactic acid determination and the buffer solution in which the spermatozoa were suspended after centrifuging from the seminal fluid

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have been described (5). The respiration studies were made at 37° with the Barcroft apparatus as in previous work (2). All the data on the oxygen uptake of spermatozoa in the presence of phospholipids are corrected for the autoxidation of the phospholipid itself which was measured in control determinations for each experiment.

Preparation of phospholipids. Fresh egg yolks were extracted twice with 2.5 volumes of acetone. The residue was extracted several times with cold ether and the combined ether extracts evaporated under reduced pressure to a small volume. Ten volumes of acetone were added and the gummy precipitate which formed was again taken up in ether, filtered and reprecipitated. This preparation—*crude egg phospholipids* (I)—was separated by means of ethyl alcohol into *crude lecithin* (II) and *crude cephalin* (III). *Purified lecithin* (IV) was prepared by treating an alcoholic solution of preparation II with alcoholic cadmium chloride and purifying the precipitated cadmium lecithinate according to the method of Levene and Rolf (6). More recently this preparation has been further purified by the procedure of Pangborn (7).

Phospholipids from rat liver (V) were prepared from the ether extract of ground, acetone-extracted rat livers by the procedure used in obtaining preparation I above.

Crude soybean phospholipids (VI) contained 70 per cent acetone insoluble lipids and 30 per cent soybean oil. Soybean "*lecithin*" (VII) and "*cephalin*" (VIII) were obtained from preparation VI by extracting with acetone until oil-free and separation on the basis of their solubilities in ethyl alcohol.[2]

All phospholipid preparations were stored under acetone in the refrigerator to prevent autoxidation. For experimental use the phospholipids were emulsified in buffer solution or 0.9 per cent saline and added at a level of 5 to 10 mgm. per cubic centimeter of the final sperm-buffer suspension.

RESULTS. *Effect of phospholipids on motility.* It is evident from table 1 that phospholipids from egg yolk and other sources were effective in promoting motility. Neutral fat and the mono-ester ethyl laurate were harmful to the spermatozoa. When stored at 10° the spermatozoa maintained their initial motility much longer in the presence of phospholipids than in the presence of added glucose only. From the data in table 2 it is evident that added phospholipid as well as the intracellular reserve of the sperm are utilized by an oxidative process since both are effective in maintaining motility only under aerobic conditions.

Effect of phospholipids on respiration. The rate and duration of oxygen consumption by bull spermatozoa were markedly increased when phospholipids were added to the suspension medium. The results of a typical

[2] We are indebted to Dr. J. L. Gabby of the Glidden Company, Chicago, Illinois, for the soybean phospholipid preparations.

experiment are shown in figure 1. It was found that the increase in respiration of spermatozoa obtained by adding phospholipids depended largely

TABLE 1

Effect of various metabolites on the duration of motility of spermatozoa stored at 10°C.

MEDIUM	METABOLITE ADDED	MOTILITY DURATION
		hours
M/15 Na-K phosphate, pH = 6.75	None	24
	Glucose	78
	Egg lecithin II	151
Modified Ringer-phosphate, pH = 6.75	None	20
	Glucose	50
	Egg phospholipids I	150
	Egg "lecithin" II	150
	Egg "cephalin" III	72
	Liver phospholipids V	150
	Crude soybean phospholipids VI	0
	Soybean "lecithin" VII	120
	Soybean "cephalin" VIII	120
	Egg neutral fat	0
	Ethyl laurate	0

Spermatozoa were centrifuged from the seminal fluid and made up to original semen volume with the buffer solution. Where glucose was added, the final concentration was 0.04 molar. Motility duration indicates the length of time a motility of 1+ or better (5) was maintained.

TABLE 2

Effect of metabolites on sperm motility in air and under nitrogen

MEDIUM, RINGER-PHOSPHATE PLUS	ATMOSPHERE	MOTILITY RATING		
		1 hour	3 hours	5 hours
None	Air	4+	1+	F.M.
	N_2	F.M.	Dead	
0.04 molar Glucose	Air	5+	4+	3+
	N_2	4+	4+	4+
"Lecithin" (II)	Air	4+	3+	3+
	N_2	F.M.	Dead	

Semen was centrifuged, the sperm taken up in 0.9 per cent NaCl, centrifuged again and finally made up to original semen volume with Ringer-phosphate (pH = 6.8). Incubated at 37°C.

on the length of time between the collection of the semen and the beginning of the experiments. This is demonstrated in table 3. Fresh, rapidly respiring spermatozoa showed only a small increase in oxygen consumption

when phospholipids were added while samples which had been held from 2 to 5 hours before beginning the respiration experiments showed considerable increases. As the spermatozoa "age" the intracellular reserves are apparently depleted and the added phospholipid is more rapidly utilized. In ten experiments the addition of phospholipids caused an av-

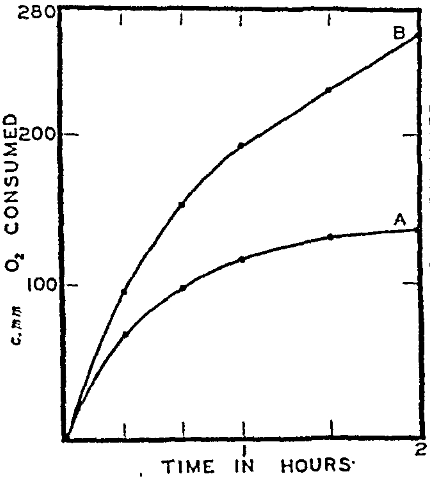

Fig 1

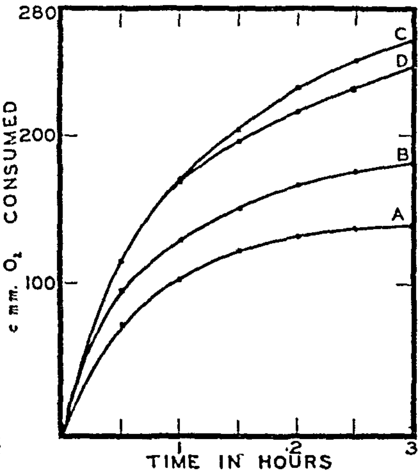

Fig. 2

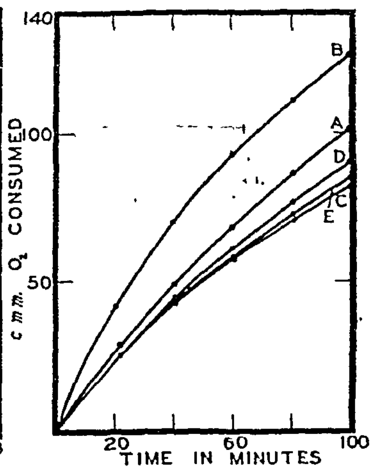

Fig. 3

Fig. 1. Effect of lecithin on oxygen consumption of bull spermatozoa. Semen which had been held at 10° for 4 hours was centrifuged and the spermatozoa were washed by suspending in modified Ringer-phosphate buffer. After centrifuging again the spermatozoa were suspended in modified Ringer-phosphate pH = 6.8. Each Barcroft flask contained one billion spermatozoa in 3 cc. buffer. A = endogenous oxygen consumption, B = oxygen consumption of spermatozoa in presence of lecithin II.

Fig. 2. Effect of ascorbic acid on the oxygen consumption of bull spermatozoa. Spermatozoa were centrifuged from semen, washed by suspending in 0.9 per cent saline and after centrifuging again were suspended in sufficient modified Ringer-phosphate buffer (pH = 6.8) to give original volume of semen. Eight-hundred million spermatozoa per flask. A, no substrate; B, with 0.001 molar ascorbic acid; C, with lecithin II; D, with 0.001 molar ascorbic acid and lecithin II.

Fig. 3. Effect of glucose and lecithin on oxygen consumption of bull spermatozoa. Semen stored 4 hours at 10° was centrifuged and the spermatozoa suspended in sufficient Ringer-phosphate buffer to give original semen volume. One and five-tenth cubic centimeter sperm suspension containing 700 million spermatozoa (= 14.1 mgm. dry cells) per flask. Final volume made up to 3 cc. per flask with 0.9 per cent saline or solution of metabolite in 0.9 per cent saline.

A, no substrate; B, with lecithin IV; C, with 0.02 molar glucose; D, with lecithin IV and 0.02 molar glucose; E, with lecithin IV and 0.04 molar glucose.

erage increase in the rate of oxygen consumption of 38 and 46 per cent above the endogenous rate for the first and second hours respectively.

Quastel and Wheatley observed an increase in the rate of fatty acid oxidation by liver slices when ascorbic acid was added (8). This observation together with the demonstration that ascorbic acid promoted the

production of viable, fertile spermatozoa in sterile bulls (9, 10) led us to try the catalytic effect of ascorbic acid on the oxygen consumption of washed spermatozoa (fig. 2). Ascorbic acid itself increased the oxygen uptake of spermatozoa by an amount sufficient to account for its oxidation to dehydroascorbic acid. However, ascorbic acid did not increase the rate of oxygen consumption of spermatozoa in the presence of lecithin.

TABLE 3

Relation of "age" of spermatozoa to oxygen consumption

TIME BETWEEN COLLECTION OF SEMEN SAMPLE AND BEGINNING OF RESPIRATION MEASUREMENT	Q_{O_2}*—FIRST HOUR	
	"Endogenous"	With lecithin VII
hours		
0.5	−9.20	−9.44
0.5	−7.45	−8.05
0.8	−7.04	−8.00
2.0	−6.44	−8.34
3.5	−4.56	−6.90
4.0	−4.27	−7.10
4.0	−4.86	−6.63

* Q_{O_2} = c.mm. O_2/mgm. dry cells/hr.; 37 to 55 million (average 40 million) bull spermatozoa yield 1 mgm. dry matter. Respiration was linear with from 100 million to 1 billion spermatozoa in 3 cc. suspension.

TABLE 4

Effect of phospholipids on lactic acid production by spermatozoa

MEDIUM, RINGER-PHOSPHATE-GLUCOSE PLUS	LACTIC ACID PRODUCED PER CC. SPERM SUSPENSION* IN 3 HOURS	
	Aerobic	Under N_2
	mgm.	*mgm.*
None	0.80	0.83
Egg phospholipids (I)	0.81	0.85

* Sperm concentration 500 million/cc.

Spermatozoa were centrifuged from semen and made up in Ringer-phosphate containing 0.04 molar glucose. Incubated at 37°C.

As shown in figure 3, the addition of lecithin did not increase the oxygen consumption of bull spermatozoa when sufficient glucose was present in the medium. This would indicate that glycolysis occurred in preference to the oxidation of added lecithin just as it does in preference to the oxidation of the intracellular reserves (2).

Effect of phospholipids on glycolysis. Phospholipids did not exert their influence on sperm motility by catalytically increasing the rate of glycolysis either aerobically or anaerobically. As shown in table 4, the amount of

lactic acid produced was the same in the presence of phospholipids as it was without added phospholipids. The fact that lactic acid production was not decreased in the presence of lecithin is further evidence that glycolysis is the preferential source of energy for the bull sperm.

DISCUSSION. The data presented show that phospholipids from egg yolk and other sources were effective in maintaining the motility and oxygen consumption of bull spermatozoa in a sugar-free medium. While it is possible that the added phospholipid exerted its effect by acting as a catalyst or by altering the physical properties of the suspension medium, the more plausible explanation is that it was used as a metabolite. Phospholipids had no effect on motility under anaerobic conditions and did not influence glycolysis under either aerobic or anaerobic conditions. If phospholipids were catalyzing the oxidation of an intracellular metabolite, one would expect an increased utilization and early depletion of this reserve with inactivation of the sperm as a result. On the contrary, lecithin was effective in maintaining the motility of bull spermatozoa for long periods of time. The comparatively poor result obtained with lecithin in previous work (4) was due to the fact that the lecithin preparation used had been obtained from dried egg yolk and was discolored from contact with air. Discolored, partially autoxidized lecithin preparations from sources other than egg yolk have also been found ineffective in promoting motility. A fresh, chemically unchanged preparation was necessary before positive results could be obtained. It is interesting to note that Milovanov found that lecithin increased the life duration of spermatozoa and recommended addition of lecithin to certain semen diluting fluids to protect the lipoid capsule of the sperm cell (11).

It is generally conceded that fats are transported across the cell membranes in the form of phospholipids. It is not surprising then to find that neutral fat or simple fatty acid esters were not utilized by the spermatozoa, for it seems entirely possible that they do not enter the sperm cell.

In metabolic studies on most tissue slices, minced or homogenized tissue suspensions, it is difficult to correlate the respiration or chemical reactions with the function of the tissue. Spermatozoa provide an excellent means of studying the significance of the measured reactions in the maintenance of the function of the cell for the chemical and respiration results can be correlated with motility or also with fertilizing ability. In the work reported here it was shown that the increased oxygen consumption of spermatozoa in the presence of phospholipids was correlated with increased and prolonged motility.

SUMMARY

1. Phospholipids from a variety of sources were effective in maintaining the motility of bull spermatozoa in a sugar-free medium under aerobic

conditions. It was shown that the effect was not due to catalysis of sperm glycolysis.

2. In the presence of phospholipids the rate and duration of oxygen consumption by bull spermatozoa was greatly increased. Ascorbic acid did not catalyze the oxidation of phospholipids by bull spermatozoa.

3. The oxygen consumption of bull spermatozoa *in a medium containing glucose* was not appreciably increased by the addition of lecithin. This, together with the demonstration that lecithin does not alter the rate of glycolysis, is further evidence that bull spermatozoa call upon oxidative processes *as sources of energy for motility* only when deprived of glycolyzable sugars.

4. The similarity in the manner in which added phospholipid and the intracellular reserves are utilized by spermatozoa is in agreement with our previous conclusion (2) that the intracellular reserves of the spermatozoa are phospholipid in nature.

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IODINE FIXATION IN THE THYROID AS INFLUENCED BY THE HYPOPHYSIS AND OTHER FACTORS

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The passage of iodine into the thyroid (1) is easily observed with the help of radio-iodine (2, 3, 4, 5, 6). In our investigation of this problem, the ability of the gland to fix iodine was found to be restricted within definite limits and to be dependent upon the activity of the hypophysis.

METHOD. The use of the isotope I^{128}, with a half-life of 25 minutes, limited the duration of the experiments to two hours. The radio-iodine was prepared by bombarding ethyl iodide with either the neutrons of radon-beryllium or those resulting from the impact of 2000 kv. x-rays on beryllium. The neutrons, producing both radioactive iodine and 2 to 6 mgm. of inactive iodine from the ethyl iodide (4 liters) made it unnecessary to add extra iodine for the purpose of "carrying" the radioactivity. The iodine was extracted as hydroiodic acid by shaking with a concentrated solution of hydrogen sulphide in a separatory funnel. The solution was separated and hydroiodic acid was titrated with sodium hydroxide in presence of methyl orange. After boiling to eliminate the hydrogen sulphide, there remained a solution of sodium (radio-)iodide of known iodine titer, which could be used in animals.

The animals were kept on standard diets (except for the guinea pigs), at 22° (±2°)C. The injections of radio-iodine were performed either in the veins (rat, rabbit) or the heart (guinea pig, mouse). Doses of 0.5 mgm. of iodine were injected per 100 grams of body weight. The animals were sacrificed by section of the carotids 30 minutes after an injection, unless otherwise indicated. For the estimation of the radioactivity in the organs, these were weighed, finely ground and evenly distributed on a rectangular aluminum foil 6 by 2.5 cm. The foil was then fastened lengthwise on a 12 by 5 cm. piece of adhesive tape and the whole covered with cellophane. The adhesive was rolled into a cylinder exactly fitting the Geiger counter in such a way as to set the aluminum in front of the active part of the counter. Less than 100 mgm. of organ was estimated at one time, three measurements being made at 25-minute intervals whenever

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possible. The results were plotted on a logarithmic scale and the best-fitting line was drawn parallel to the slope of the decay of I^{128}. The radio-iodine content was estimated by comparison with the curve furnished by the original radioactive solution, a sample of which had been diluted, put on aluminum, evaporated at 60°C. and measured on the counter in the same way as the organs. The amount of iodine which had been fixed by the organs could thus be calculated. As the justification of chemical and other techniques will be reported separately, it may suffice to state that the accuracy of the determinations was variable, being much reduced in the longer experiments, and that, with the exception of the diiodotyrosine experiment, the limit of error was found to approximate ±10 per cent.

PRELIMINARY EXPERIMENTS. The fixation of greater amounts of iodine by the thyroid than by other tissues already observed in dogs (1), rabbits (2, 4) and humans (3), is confirmed here in four animal species (table 1). The most regular results were found in the rat thyroid (smallest probable

TABLE 1

Concentration of radio-iodine in the organs thirty minutes after a single injection of Na(radio-) iodide

	CONCENTRATION OF RADIO-I IN THYROID (MGM. PER 100 GM. OF FRESH TISSUE)	CONCENTRATION OF RADIO-I IN LIVER (MGM. PER 100 GM. OF FRESH TISSUE)
Guinea pig	16.2 ± 1.8	0.3
Rat (Wistar)	8.1 ± 0.6	0.5
Mouse (CBA)	7.8 ± 1.5	0.4
Rabbit	14.3 ± 3.2	0.3

Each group was composed of 6 animals.

error), but the greatest fixation of iodine was observed in the thyroid of the guinea pig.[2] The rapidity of the fixation of iodine by the thyroid (1, 2) is illustrated by the experiment reported in figure 1. In addition, these results show that with the dose of iodine used, the concentration of iodine in the thyroid reached a maximum within six minutes after the injection and then remained at a constant level.

In the organs and body fluids not reported in the table, the values were of the same order as in the liver, although very irregular, as indicated by Ariel et al. (5), and without any outstanding concentration of iodine, except in the urine and especially in the gastric juice (6). A chemical separation of the gastric juice of unfed animals, with or without histamine treatment, showed that the iodine behaved like chlorine, since one fraction was in the form of hydroiodic acid, distilling upon gentle heating and precipitating in the distillate after addition of acid silver nitrate; while

[2] In two rabbits, the iodine collection was greater than in any guinea pig.

the other fraction consisted of iodide which remained in the distillation residue wherefrom it could be precipitated out as silver iodide, in nitric medium.

The possibility that other iodized compounds might yield their iodine to the thyroid more readily than the iodides, led to an investigation of the fate of some iodate and diiodotyrosine synthesized from radio-iodine. The use of these compounds presupposed that the iodine atoms present in their molecule could not be exchanged with the iodine atoms of the blood iodide. This possibility was investigated by mixing *in vitro* a solution of radioactive iodide and solutions of more complex iodized compounds as indicated in table 2, in order to see whether or not the radio-iodine would enter the iodate or diiodotyrosine molecule. The results showed the absence of exchange since, within 30 minutes of contact, no radioactivity

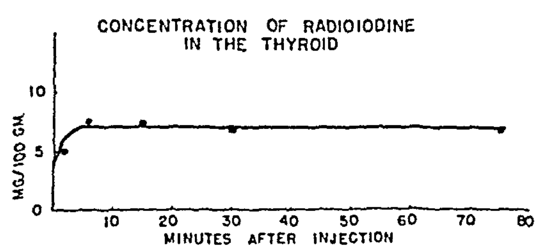

Fig. 1

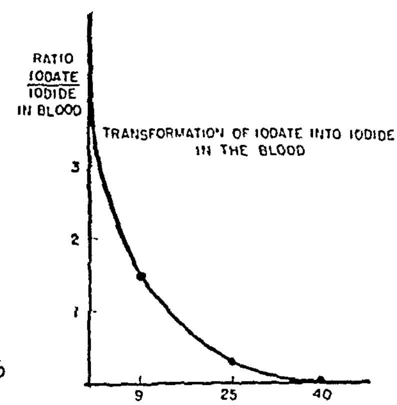

Fig. 2

Fig. 1. Fixation of radio-iodine in the rat thyroid at various intervals after an injection of 0.75 mgm. of radio-iodide. Each dot corresponds to an average of 6 animals.

Fig. 2. Ratio of iodate I to iodide I in the blood at various intervals after an injection of 0.75 mgm. of radio-iodate. Time is indicated in minutes on the abscissa.

that could not be accounted for by experimental errors was exchanged between the iodide and the other compounds examined.

Radioactive iodate, obtained by Baxter and Butler's method (7), was injected intravenously at the dose of 0.75 mgm. of radio-iodine into rats of about 150 grams, in 3 experiments including six rats each. The animals were sacrificed in groups of two at time intervals of 9, 25 and 40 minutes, respectively, after the injection. The amount of radio-iodine in the thyroid averaged 0.9 mgm. and 2.9 mgm. per 100 grams of fresh tissue, respectively, 9 and 25 minutes after the injection; 40 minutes after the injection, the iodine could not be measured any longer, since the radioactivity was then exhausted. The fact that the amount of radio-iodine in the thyroid was small and continued increasing after the ninth minute after injection could be partly explained by an analysis of the fate of the

iodate in the blood stream. This analysis was carried on after the addition of inactive iodide and iodate to the blood to serve as carriers of the suspected radioactive molecules. After trichloracetic treatment of the blood and washing of the precipitate, the subsequent precipitation of the iodate as barium salt and of the iodide as silver salt showed that the radio-iodate decreased while the radio-iodide increased progressively. Therefore in the blood the iodate was transformed into iodide to such an extent that 40 minutes after injection of the iodate only traces of it were still present (fig. 2). The amount of radio-iodine in the thyroid appeared to increase simultaneously with the increase of the amount of iodide in the blood. This suggested that the iodine present in the iodate molecule entered the thyroid only after its transformation into iodide had taken place in the blood.

TABLE 2

Exchanges between iodate or diiodotyrosine or thyroxine and iodine ion of Na radio-I

SUBSTANCES ADDED TO Na RADIO-I	MEDIUM	TOTAL NUMBER OF COUNTS IN NaI AT BEGINNING	DURATION OF CONTACT	PERCENTAGE OF ACTIVITY IN IODATE DIIODOTYROSINE OR THYROXINE AT THE END
			minutes	
Iodate	Water	105,000	30	0.002
Iodate	Boiling water	107,000	30	0.0002
Diiodotyrosine	Water	106,000	30	0.18
Diiodotyrosine	Water	614,000	8	0.05
Diiodotyrosine	Blood	174,000	15	0.06
Thyroxine	Water + 20% of NH_3	406,000	90	0.27
Thyroxine	Water + 20% of NH_3	252,600	30	0.08
Thyroxine	Water + 20% of NH_3	255,000	30	0.50

Similar although less precise results were obtained with radioactive diiodotyrosine, prepared by Oswald's method (8). Relatively high doses of this compound, corresponding to 5 mgm. of iodine per 100 grams of body weight, were given intravenously to each animal in 2 groups of 4 rats each. The time required for the preparation of radioactive diiodotyrosine and its subsequent use in animal experiments precluded exact determinations. With this limitation, the results (table 3) showed, as in the case of the iodate, that the fixation of the radio-iodine in the thyroid was not immediate, and probably followed the rapid transformation of diiodotyrosine into iodide, as observed in blood and urine. In the literature, it has been reported that the transformation of diiodotyrosine into iodide is only partial (9); our different results may be due to the smaller doses of diiodotyrosine used here.

These results were sufficient to warrant the exclusive use of the iodides

in future experiments, since other iodized compounds appeared to be transformed into iodide before their iodine was taken up by the thyroid.

Response of the thyroid to various doses of iodine. In order to test the efficiency of the thyroid gland, doses of iodine varying from 30 to 9000 micrograms were injected into guinea pigs weighing 150 (±20) grams. The results (fig. 3) show that the thyroid fixes increasing amounts of iodine when the doses of iodine are raised. However, if the amount of radio-iodine in the gland is expressed in per cent of the total radio-iodine injected, it can be seen that, with injections of 30, 300 and 9000 micrograms

TABLE 3

Effects of injection of 10 mgm. of radioactive diiodotyrosine

TIME BETWEEN INJECTION AND AUTOPSY	CONCENTRATION OF RADIO-I IN THYROID	PERCENTAGE OF DIIODOTYROSINE IODINE TRANSFORMED INTO IONIZED IODINE	
		Urine	Blood
minutes	*mgm. per 100 gm.*	*per cent*	*per cent*
10-16	<8.5	90	79
30	12.6	91	97.5

of iodine, a thyroid fixation of 1, 0.3 and 0.07 per cent of the total respectively takes place. The smaller the dose of iodine injected, the greater is the proportion of iodine entering the gland. The efficiency of the thyroid is at its best with the smallest doses of iodine. In experiments with smaller or physiological (tracer) doses of iodine, Hertz (10) and Mann (private communication) found that a much higher percentage of iodine was taken up by the gland.

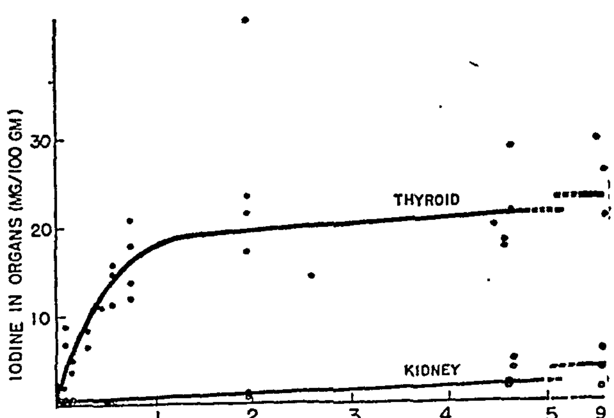

Fig. 3. Fixation of radio-iodine in the guinea-pig thyroid (dots) and kidney (circles) thirty minutes after injection of various doses of radio-iodide.

If the amount of injected iodine was increased beyond 750 micrograms (a dose which happened to be equal to our standard of 0.5 mgm. per 100

grams of body weight), the concentration of 16 mgm. of radio-iodine in the guinea pig thyroid could be increased only slightly even when considerably higher doses were injected (fig. 3). This slow increase of thyroid iodine was similar to what took place in the other organs examined (kidney and stomach wall), where one observed only a slight progressive increase in direct relation to the quantities injected, probably due to a gradual overflooding with iodine. Therefore, with the higher doses, the specific capacity of the thyroid for iodine is exhausted.

However, the curve that indicates this "saturation" of the thyroid (fig. 3) might be simulated by a curve due to exchange equilibria between the iodine atoms of the blood and those of the thyroid. Such an exchange might occur in either of two possible ways. The radio-iodide injected into the blood might exchange with the iodine atoms of diiodotyrosine, or thyroxine, which are the only organic iodized compounds in the thyroid (11). However it has been shown (table 2) that the iodine of these compounds is not exchangeable, at least in 30-minute experiments. As an alternative possibility, the blood radio-iodine could exchange with the small amount of ionized iodine atoms (11) present in the thyroid. This latter possibility was tested by injecting radio-iodide to animals having received a few hours previously some ordinary iodide. If the entrance of iodine into the thyroid were an exchange phenomenon, it should proceed regardless of whether or not a previous injection of iodine had been given. However, when an injection of radio-iodine was given after injections of chemical iodine, only a small fraction of the expected uptake of radio-iodine could be found in the thyroid (table 4, groups 1 and 2). Such results, as already pointed out by Hertz et al. (2), can be explained only by the assumption that some new iodine was added to the gland, with no or little exchange taking place. Indeed Marine and Rogoff using chemical methods have shown the existence of an addition of iodine to the gland after iodide injection, but their experiment did not rule out the possibility of a simultaneous exchange (1). It may be concluded that, after an addition of about 16 mgm. of iodine per 100 grams of thyroid (guinea pig), this gland reaches a state of saturation. In a saturated thyroid, it is not possible to produce an important increase of the iodine concentration by the injection of either large (fig. 3) or repeated (table 4, groups 1 and 2) doses of iodine.

Exchanges being ruled out, at least for the most part, the saturation could be due to a chemical process, since the iodine entering the thyroid could react with free chemical linkages, which, being then saturated, would not be able to fix additional iodine. In order to decide whether the radio-iodine in the thyroid had undergone some chemical transformation or was still in the form of iodide, chemical analysis of the gland was performed thirty minutes after a standard injection of radio-iodine into guinea pigs.

The tissue was ground and extracted with hot alcohol; and after filtration and evaporation of the filtrate, the residue containing all the radioactivity was dissolved in alkaline solution, precipitated with trichloracetic acid and filtered. About 90 per cent of the total radioactivity was in the filtrate, where it could be precipitated as iodide with acid silver nitrate, the rest of the radioactivity being in the trichloracetic precipitate with the thyroglobulin. Hot water extraction, dialysis and electrolysis in acid medium on a copper plate, also indicated that at least 90 per cent of the radio-iodine was still present in the thyroid as iodide, while it seemed as though a fraction less than 10 per cent had been incorporated into the molecule of thyroglobulin. Therefore the entrance of iodine into the thyroid was not the direct consequence of a chemical reaction; its accumulation

TABLE 4

Fixation of radio-iodine in thyroid of animals having received previous injections of inactive iodine

EXPERIMENT NUMBER	AMOUNTS OF IODINE INJECTED BEFORE RADIO-ACTIVE IODINE	INTERVAL BETWEEN LAST INJECTION OF IODINE AND RADIO-ACTIVE IODINE	AMOUNT OF FIXED RADIO-ACTIVE IODINE (IN MGM. PER 100 GM. OF FRESH TISSUE)	
			Thyroid	Liver
1	0	(controls)	6.7	0.4
	1 mgm. daily for 8–10 days	5 hours	1.8	0.3
2	0	(controls)	8.4	0.2
	1 mgm. daily for 8–10 days	8½ hours	1.1	0.1
3	0	(controls)	11.8	0.4
	1 mgm. daily for 8–10 days	48 hours	3.2	0.3
4	0	(controls)	8.7	0.3
	1 mgm. daily for 8–10 days	72 hours	6.8	0.4

in this instance would seem to be more in the nature of a process of absorption.

Although the short life of the radio-iodine prevented an analysis of its transformation in the thyroid over a period longer than 30 minutes, an indirect method gave some information as to the ultimate fate of the fixed iodine. Since a thyroid lost its ability to fix iodine when saturated, an experiment was designed to determine the time required for the restoration of a normal iodine fixation. A preliminary experiment, performed a week after saturation of the thyroid with inactive iodine, showed that the gland had at that time completely recovered its ability to fix iodine. Eight groups of five rats weighing about 150 grams were then subdivided into 4 control groups and 4 groups receiving 1 mgm. of iodine in the form of

iodide daily for 8 to 10 days, this dose being insufficient for a reduction of the thyroid activity of the animals as judged from the histology of the gland. A single injection of 0.75 mgm. of radio-iodine as NaI was then administered to the control animals, while the treated animals were given the same dose at various intervals after their last injection of inactive iodide (table 4). The results indicated that three days after the last injection of inactive iodide, the iodine-fixing capacity of the thyroid had almost returned to normal (fig. 4). The time required for the restoration of the ability of the gland to fix iodine may indicate that the amount of iodine necessary to saturate the gland was utilized in a little more than three days.

Rôle of the hypophysis. Hypothyroidism was produced in the rat by hypophysectomies performed by A. Chamorro (4). This operation re-

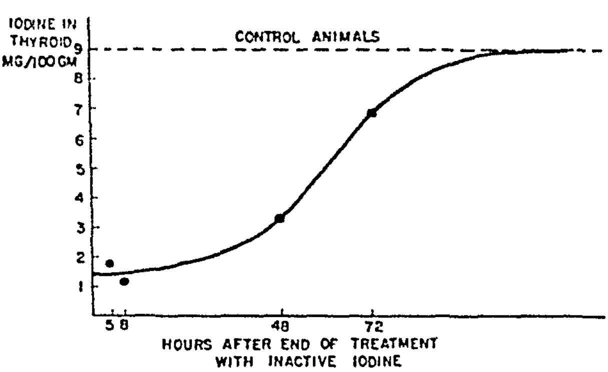

Fig. 4

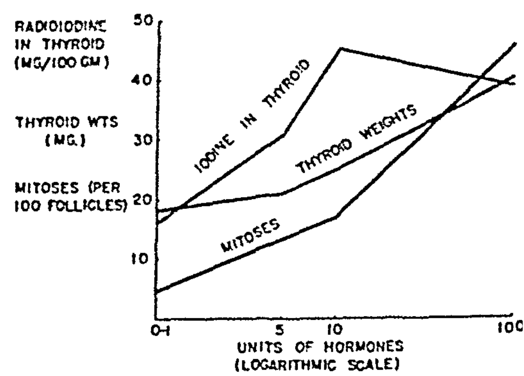

Fig. 5

Fig. 4. Fixation of radio-iodine in the rat thyroid when 0.75 mgm. of radio-iodide was injected at various intervals after saturation of the thyroid.

Fig. 5. Effect of injection of 5, 10 and 100 units of thyrotropic hormone on the weight, mitotic activity and iodine-fixing ability of the guinea-pig thyroid.

sulted in a decrease of the ability of the thyroid to fix iodine (table 5). The decrease was not very sudden, however, since 5 days after removal of the hypophysis, the fixation of iodine by the thyroid was only slightly reduced. At 116 days after the operation, the concentration of iodine in the thyroid was very small, although still greater than that of other organs, such as the liver.

The decrease in the concentration of radio-iodine after hypophysectomy could be due to a relative decrease of active thyroid tissue, since the operation produces an accumulation of colloid with a reduction in the size and probably in the number of the cells. Another alternative was that the remaining cells had lost their efficiency in fixing iodine. The relative amounts of cells and colloid were estimated on histological sections of the thyroids in two animals operated 20 days previously and their two controls, as also in two operated 116 days previously and their two controls. Cam-

era lucida drawings of 4 wide (Reichert) fields on thyroid sections at a magnification of 150 × were performed on each animal. By cutting off and weighing the cells and colloid of the drawing, it was found that the average ratio of colloid to cells had increased from 0.69 to 3.13 in the twenty days following hypophysectomy and from 0.62 to 4.7 in the four months following this operation. Simple equations deducted from these figures showed that, 20 and 116 days after the operation, the relative amount of cells had decreased by factors of 0.41 and 0.28 respectively. Since the radio-iodine concentration in the thyroid had decreased by factors of 0.42 and 0.11 respectively in these two groups (table 5, last column), it can be seen that this reduction in the iodine uptake was comparable to the reduction in the amount of thyroid cells.[3] If, therefore, the thyroid

TABLE 5

Fixation of iodine by rat thyroid after hypophysectomy

NUMBER OF DAYS AFTER OPERATION	NUMBER OF ANIMALS	AVERAGE BODY WEIGHT		AVERAGE WEIGHT OF THYROID	PER CENT OF INJECTED RADIO-ACTIVITY IN THYROID	CONCENTRATION OF RADIO-IODINE (MGM. PER 100 GM. OF FRESH MATERIAL)		RATIO OF THYROID RADIO-I CONCENTRATION: TREATED / CONTROLS
		Before operation	At autopsy			Thyroid	Liver	
		grams	*grams*	*mgm.*				
0	5	156	157	12	0.12	7.2	0.3	
6	5	142	129.3	9	0.08	6.8	0.4	*0.94*
0	1	82	87	9	0.10	8.7		
9	1	82	56	8	0.03	4.3		*0.49*
0	2		136	13	0.15	9.1	0.4	
20	2		60	7	0.03	3.8	0.6	*0.42*
0	5	131	196	18	0.24	11.7	0.5	
116	7	114	109	11	0.02	1.25	0.4	*0.11*

of the hypophysectomized animal fixed less iodine, it was due mostly to the small amount of thyroid tissue present in these thyroids, although a slight decrease in the efficiency of the remaining cells was not excluded, especially four months after the operation.

Hyperthyroidism, obtained through injections of a highly purified Schering thyrotropic hormone into guinea pigs (8 injections in 4 days), produced a marked increase in iodine uptake by the thyroid (table 6). The increase in the concentration of radio-iodine could be due either to the relative increase of active thyroid cells following the departure of colloid, or to an increase in the efficiency of the cells, or to both factors. In

[3] It was assumed for these calculations that the changes in the connective tissue were negligible. This implication involved only a small error.

three control and three guinea pigs treated with 10 units of thyrotropic hormones, the ratio of colloid to cells was estimated as above. The decrease of these ratios from 0.80 to 0.43 under the influence of the injections implied that the relative amount of colloid had decreased by a factor of 1.48 and the proportion of cells had increased by a factor of 1.26. On the other hand the concentration of radio-iodine in the thyroid increased, after injection of 10 units of hormone, by a factor always superior to 2, reaching 4.2 in one group (table 6, last column). The increase in the "cell concentration" could account for only a small part of the increase in radio-iodine concentration.[3] Therefore the cells must have been functioning more efficiently when under the influence of thyrotropic hormone. In other words, the thyrotropic hormone had increased the capacity of the thyroid gland for iodine, partly by reducing the amount of colloid and thus increasing the cellular content, but mostly by increasing the activity of the cells.

TABLE 6

Fixation of iodine by guinea pig thyroid after thyreotropic hormone

NUMBER OF EXPERIMENT	UNITS OF HORMONE IN 4 DAYS	NUMBER OF ANIMALS	AVERAGE THYROID WEIGHT	PER CENT RADIO-ACTIVITY IN THYROID	CONCENTRATION OF RADIO-IODINE (MGM. PER 100 GM. OF FRESH MATERIAL		RATIO OF THYROID RADIO-I CONCENTRATION: TREATED / CONTROLS
					Thyroid	Liver	
			mgm.				
1	0	5	21	0.3	13.5	0.2	
	10-12	6	39	2.1	43.3	0.1	3.2
2	0	3	20	0.3	11.8	0.4	
	10	3	21	1.3	51.4	0.4	4.3
	100	3	36	2.4	49.6	0.5	4.2
3	0	3	21	0.5	18.0	0.3	
	10	2	26	1.5	43.2	0.2	2.4
	100	3	44	1.8	35.9	0.3	2
4	0	2	21	0.4	14.3	0.1	
	5	3	23	0.9	30.0	0.2	2.1
	10	2	25	1.3	37.6	0.2	2.6
	100	2	42	2.1	35.3	0.1	2.4
5	0	2	11	0.3	21.1	0.1	
	2	3	19	0.7	26.9	0.1	2.0
	5	2	20	0.9	34.4	0.1	2.8
	100	2	40	1.6	32.4	0.1	2.4

The previous analysis of the effects of thyrotropic hormone was based upon results in animals receiving injections of 10 units of this hormone

in 4 days. Increasing the amount of hormone to 100 units, however, did not produce further concentration of iodine in the thyroid (table 6). The understanding of these results was facilitated by plotting them on a logarithmic scale (fig. 5), along with the average weights of the glands and the average number of colchicine mitoses per 100 follicles. The mitoses were counted on 5-micron sections in 300 thyroid follicles in each of 18 guinea pigs divided into groups of 6, the groups receiving 0, 10 and 100 units of thyrotropic hormone respectively over a period of 4 days. The animals were given colchicine 9 hours before autopsy (12). Increasing the dose of thyrotropic hormone from 10 to 100 units produced a considerable growth of the thyroid as shown by the weight and mitosis curves, but the radio-iodine concentration decreased. The radio-iodine concentration was smaller with 100 units than with 10 units of thyrotropic hormone in each case (table 6, 5th column), thereby indicating not only that the efficiency of the thyroid tissue could not be increased over the limit reached with 10 units of hormone, but that the newly formed thyroid tissue, testified to by the mitoses and weight increase, could not be stimulated as effectively as the original thyroid tissue.[4] It is a frequent occurrence in biology that the function of rapidly growing tissue is somewhat hampered by the growth itself, as shown for instance by Doljanski (13).

Discussion. Intravascular administration of sodium iodide was followed by a deposition of iodide as such in the thyroid gland. The amount of iodide that could be collected by the gland was limited in the rat to about 10 mgm. per 100 grams of fresh tissue and in the guinea pig to nearly 20 mgm. per 100 grams of fresh tissue. These values could be but slightly increased by injecting larger or repeated doses of iodine, and therefore indicated the extent of the capacity of the thyroid for ionized iodine.

Some of the results (fig. 4) suggested that the amount of iodide necessary for saturation of the thyroid gland was utilized in a little more than 3 days. The amount used up in 3 days could be roughly estimated by the difference between the figures obtained for the radio-iodine concentration in the thyroid of the treated animals in groups 1 and 4 (table 4), that is to say, 5 mgm. per 100 grams of fresh tissue. The rapidity with which this iodine stored in the thyroid may be utilized was unexpected, and points to the necessity of a regular iodine intake.

When by hypophysectomy the source of thyrotropic hormone was eliminated, the radio-iodine and the cellular content of the thyroid decreased in the same proportion. It thus seems that, after the operation, the remaining cells, in spite of their atrophy, can fix as much iodine as an equal cytoplasmic volume of normal cells. Therefore the operation does not affect the specific iodine-fixing ability of the thyroid tissue, although a slight decrease of this activity was not excluded by the present data.

[4] There is a possibility that higher doses of iodine were required in this case.

Injections of thyrotropic hormone increased the iodine fixation in the thyroid by double mechanism. An increased proportion of thyroid cells was produced and the efficiency of these cells was increased. The latter result shows the possibility for thyrotropic hormone to influence the efficiency of the cells. However, when no extra dose of this hormone is given, the level of activity of the thyroid cell is quite stable and is not much influenced by the suppression of thyrotropic hormone, as afforded by pituitary removal.[5]

CONCLUSIONS

1. The specific ability of the thyroid to fix iodine has been confirmed.
2. Experiments with radioactive iodate and diiodotyrosine suggest that only ionized iodine can be withdrawn from the blood stream by the thyroid gland.
3. The smaller the dose of iodine administered, the more efficiently it is fixed by the thyroid.
4. When doses of half a milligram or more of iodine per 100 grams of body weight are injected intravascularly, the thyroid becomes saturated with iodine and is no longer able to fix iodine selectively. In other words, the capacity of the thyroid for iodine is limited.
5. Exchange phenomena or chemical transformations play little or no rôle in the penetration of ionized iodine into the thyroid.
6. After being saturated with iodine, the thyroid recovers its normal ability to fix iodine in a little more than 3 days.
7. The extent of the decrease in iodine fixation after hypophysectomy and of its increase after injection of thyrotropic extract indicate that the hypophysis does not influence the specificity of the thyroid activity, but regulates the fixation of iodine by controlling to some extent the amount and the efficiency of the thyroid tissue.

We wish to acknowledge our debt to Prof. F. Joliot-Curie for having made possible the use of radio-iodine and for his numerous suggestions in the physical, chemical and biological fields. We also express our appreciation to Dr. K. E. Mason and Dr. S. L. Warren for the facilities afforded during the writing of the manuscript, and to Dr. V. E. Emmel for his assistance in its preparation.

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METHODS FOR THE COLLECTION OF FLUID FROM SINGLE GLOMERULI AND TUBULES OF THE MAMMALIAN KIDNEY[1]

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In 1921 Wearn and Richards (1) demonstrated the possibility of collecting fluid from single glomerular capsules in the living frog's kidney. In the 20 years which have succeeded this demonstration, methods have been gradually developed for the collection of fluid from single tubules of the amphibian kidney and for the quantitative analysis of the minute amounts of fluid obtained. The body of evidence accumulated by the application of these techniques has lent clear and decisive support to the filtration-reabsorption theory of urine formation (2). Glomerular fluid has been shown to have the composition of an ultrafiltrate of blood plasma in the eleven respects in which it has been examined. Reducing substances, chlorides and fluid have been shown to be reabsorbed from this filtrate as it passes through the tubules, and the *locus* of these reabsorptive processes has been established. The extension of this type of experimentation from the amphibian to the mammalian kidney has seemed desirable, not alone because the truth of the filtration-reabsorption theory could again be subjected to thorough examination, but also because the functional and anatomical differences between kidneys of the two types made it certain that new information would be disclosed. Progress has been made in this extension and the present paper describes methods which have proven successful in collecting fluid from single glomeruli and tubules of the mammalian kidney.

Preparation for visualization of the kidney surface. A majority of the experiments have been performed on guinea pigs and rats. In both species, a unilateral (right) nephrectomy was done from 5 to 43 days before the experiment in order to produce enlargement of the nephrons in the remaining kidney. This operation was performed on 89 guinea pigs and 56

[1] The expenses of this work have been defrayed in large part from a grant by the Commonwealth Fund of New York. A preliminary report of the investigation was made to the American Physiological Society in April, 1941 (This Journal 133: 480, 1941).

rats under aseptic precautions and with ether anesthesia. If it is assumed that the weight of the two kidneys was originally the same, hypertrophy of the remaining kidney was apparent by the fifth day post-operative, became maximum during the third week when it averaged 71 per cent in the rats and 62 per cent in the pigs, and remained stationary after that time. The subsequent procedures differed somewhat in the two species and must be described separately.

Guinea pigs. The guinea pigs, adult females weighing between 400 and 600 grams, were injected intraperitoneally with 0.45 cc. (29 mgm.) of sodium pentobarbital. Ether was given when necessary during the preparation. Sodium barbital, chloralosane and urethane were substituted for pentobarbital in a few experiments. The animal holder was a copper plate embedded in a sheet of cork and heated from beneath by a 15-watt bulb; the animal's body lay on the plate, and the cork provided attachment for the retractors and for the pins which restrained limbs and head. The trachea was isolated for the subsequent insertion of an 18-gauge needle, permitting insufflation with oxygen, and the right jugular vein was cannulated and connected with a burette containing a solution of 0.9 per cent sodium chloride or 10 per cent sucrose. Blood pressure was not usually measured. After the abdomen was opened by a mid-line incision, the entire gastro-intestinal tract was removed following successive ligation of the coeliaco-mesenteric axis, inferior mesenteric artery, esophagus, and gastro-hepatic omentum. The urinary bladder was emptied. Fat and peritoneum were removed from the lower pole of the kidney surface. The renal capsule was usually left *in situ* since it did not interfere with visibility or puncture of the surface units and since, if it were removed, the visible blood vessels became dilated, the surface covered with a layer of protein-rich fluid, and puncture was more apt to result in tears of the tubule walls or hemorrhage from the vessels which border them. The abdominal wall was retracted and raised, and the abdominal cavity filled with liquid petrolatum (light), warmed to body temperature, which covered the kidney to a depth of about 5 mm. This oil prevented the kidney from drying, aided in the diffusion of light, and could be readily distinguished from the watery fluid that originated in the nephrons. Its temperature remained between 35° and 39°C. during the experiment.

The surface of the kidney was illuminated by a 300-watt bulb focused on the butt end of a lucite rod 12 inches in length, 0.5 inch in diameter and ground down at one end to a bevelled tip 0.08 inch in diameter. When this tip was brought into contact with the oil by a micromanipulator, a considerable portion of the kidney surface was brilliantly illuminated but pulsatile and respiratory excursions made it impossible to visualize details of structure. Movements of the former type were diminished by exerting downward and lateral pressure on the upper pole of the kidney with a glass

rod, terminating in a disc one-half inch in diameter and shaped to fit the kidney surface; if the movements were still too gross, a small area could be immobilized by exerting downward pressure with the lucite rod at a point close to the glass disc. The movements due to respiration were particularly troublesome in the guinea pig and after attempts to inactivate the diaphragm and to fix or support the kidney proved unsuccessful, recourse was had to paralyzing the respiratory muscles with curare. One milligram of curare,[2] injected subcutaneously, abolished movements within

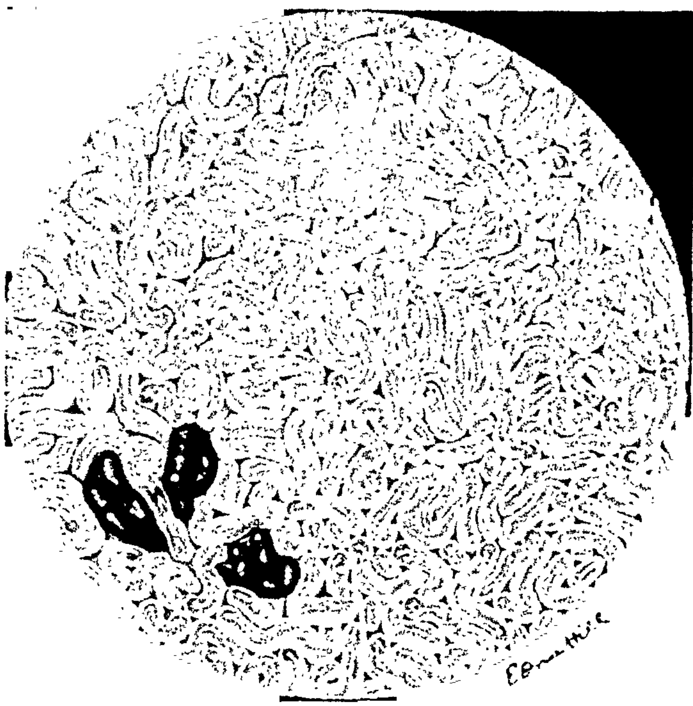


Fig. 1. Appearance of the ventral surface of a guinea pig's kidney when observed by the methods described in the text. India ink has been injected into a single tubule segment and has filled the 3 coils of a proximal convolution shown at 7 o'clock. The rounded interruption of the tubule pattern at 12 o'clock is a glomerulus. Photograph of a drawing made from life by Miss Edna Hill through the courtesy of the Harrison Department of Surgical Research, University of Pennsylvania Medical School. Magnification, approximately 50X.

ten minutes and oxygenation was maintained by intra-tracheal insufflation with 100 per cent oxygen. Under these circumstances the kidney was completely immobile and, examined with a binocular microscope at 85 magnifications, presented the appearance illustrated by the drawing of figure 1.

During the first hour after the preparation was completed, the kidney

[2] The specimen employed was supplied by the courtesy of Dr. Hans Molitor of the Merck Institute for Therapeutic Research.

continued to form urine at the rate of about 1 cc. per hour and retained its ability to reabsorb glucose and fluid, for the urine was practically free of fermentable reducing substances and contained exogenous creatinine in concentrations averaging 47 times that in blood plasma. Urine and blood specimens were collected at the beginning and end of each experiment by direct puncture of the bladder and vena cava with glass capillary pipettes containing, in the latter instance, an anticoagulant.

The chief disadvantage of the guinea pig preparation was the brevity of the period during which normal kidney function persisted. Within 1.5 hours after respiration was arrested the surface tubules were no longer distended with fluid, their epithelial lining became white and more readily visible, and the surface blood vessels showed brief cycles of contraction and relaxation (about 5 and 15 sec. respectively) which were apparently due to concurrent changes in some major renal vessel since they were accompanied by gross alterations in kidney size. When the blood pressure was measured at this time it was usually found to have fallen markedly. A second disadvantage was the considerable decrease in plasma concentrations of glucose and exogenous creatinine which occurred during the period of observation. This decrease, in the case of glucose, was especially marked after phlorhizin administration and might have been anticipated from the exclusion of the hepatic circulation but, in the case of creatinine, its mechanism was less clear.

Rats. The rats, adult males weighing between 300 and 400 grams, were starved for 18 hours preceding the experiment. They were anesthetized by the esophageal injection of 2.0 grams of urethane per kgm. in 10 cc. of tap water. The trachea was isolated, the jugular vein cannulated, and the abdomen incised in the mid-line as in the guinea pigs. The viscera were not removed but simply withdrawn from the vicinity of the kidney by retractors. The bladder was emptied, the abdominal wall supported, the abdominal cavity filled with oil and the kidney illuminated as in the guinea pigs. Blood specimens were taken by cutting the tip of the animal's tail. It proved unnecessary to arrest respiratory movements; if they were troublesome, the intravenous injection of 100 mgm. of sodium barbital usually diminished them to a point where they, as well as the pulsatile movements, could be controlled with pressure by the glass disc and lucite rod. This preparation was superior to that of the guinea pig in many respects. The entire operation could be completed in 15 minutes, the kidney was not touched, the concentrations of glucose and exogenous creatinine in blood plasma remained relatively constant, blood could be obtained at any time during the experiment, and the animal's condition continued good for 3 hours or more. A single advantage was retained by the guinea pig. In that species alone have we been able to see functioning glomeruli.

Collection of fluid from glomeruli. It seemed essential to the success of this investigation that we obtain at least a few specimens of glomerular fluid. Persistent attempts were therefore made to visualize glomeruli on the kidney surface of a variety of animals. The earlier of these attempts[3] amply confirmed Bowman's statement, made in 1842, that "the Malpighian Bodies are rarely if every visible quite on the surface of the kidney" (4). The topographical relationship of typical superficial nephrons to the kidney surface is shown in figures 6 to 10 (insets) where the glomeruli are seen to be covered by several layers of proximal convolutions. Even in the skunk, where glomeruli often lie within 0.2 mm. of the kidney surface, they are invisible by our methods of examination. In the guinea pig, however, we have had some measure of success.

In about one out of four guinea pigs, when the entire anterior surface of the kidney was examined, one or more round objects were seen which proved to be glomeruli. In the rare instances when these glomeruli lay

[3] The kidney surface of the following animals has been examined during life in an attempt to visualize glomeruli: bats (2), adult cat (1), cat aged one month (1), ferret (1), adult white mouse (1), new-born white mice (4), deer-mouse (1), muskrat (1), adult opossums (25), pouch opossums from 2 to 7 weeks after entering the pouch (4), rabbits (8), adult rats (56), new-born rat (1), skunks (7), and gray squirrel (1). No glomeruli were observed in any of these animals except in one opossum kidney within an area of focal nephritis and in one skunk kidney which was examined postmortem. Large round objects were seen in the younger pouch opossums but histological examination did not suggest that these were functioning glomeruli. We varied our method of illumination by placing the lucite rod beneath the kidney, as is possible in the bat, by thrusting it into the kidney substance and into the cysts which occasionally occur in the opossum kidney; no one of these alterations made the glomeruli visible though, in many of the animals examined, some were demonstrated to lie within a millimeter of the kidney surface. Two further variations in technique were employed in skunks, opossums, and rabbits. In the first we attempted to color the glomeruli so that their visibility might be improved; the abdominal aorta below the renal arteries being ligated and the coeliac axis cannulated, a 0.1 per cent solution of Janus Green B was injected during momentary arrest of the renal circulation; although the glomeruli proved to be well colored when the kidneys were sectioned, they were not visible from the kidney surface. Finally it was determined to expose the sub-surface glomeruli by removing a slice of kidney tissue. Either at a preliminary operation or at the time of the experiment, a thin section was removed from the kidney surface by a razor, hemorrhage being arrested by momentary clamping of the renal artery and by the application of the cut edge of a piece of skeletal muscle. A large number of glomeruli became readily visible but we were unable to collect any fluid from them and they did not appear to be functionally active. The glomerular capillaries were dilated, the corpuscles within them stagnant, and this appearance did not change when the renal blood flow was interrupted or when adrenalin or sucrose was injected intravenously. Moreover, the cut surface of the kidney was covered with a layer of exudate or tissue fluid. The technique was discarded as being grossly unphysiological, a decision which may have been premature since Ellinger (3) has described the appearance of fluorescent dyes in sub-surface glomeruli of rats exposed by an identical procedure.

completely on the kidney surface, the individual capillaries could be identified and blood could be seen flowing through them. We have only seen six such glomeruli in nearly 100 animals. There was no visible clear capsular space about them, as is the case in amphibia, and when they were punctured by a quartz pipette a capillary was torn with consequent hemorrhage from the tuft followed by permanent stasis. On one occasion puncture was accomplished without hemorrhage but apparently resulted in contraction of the afferent vessel, for all of the capillaries emptied themselves of blood and remained contracted. No one of these glomeruli showed intermittence of blood flow, but only six were observed and no one of them for over 5 minutes. The majority of glomeruli seen were less clearly visualized but proved more suitable for our purpose. They appeared as rounded reddish objects, either below the kidney surface and covered by a single layer of tubules, or as a structureless interruption in the pattern of surface tubules (fig. 1). These glomeruli could usually be punctured without hemorrhage and, in 7 experiments, sufficient fluid has been collected from them for analysis. The puncture was performed with a quartz pipette, about 7μ in internal diameter at its tip, attached to a glass rod and micromanipulator in the fashion which has been described (1). The pipette was filled with mercury except at its extreme tip, where a small quantity of a light oil colored with Scharlach-R had been introduced. When the glomerular capsule had been penetrated, the oil was injected; at first it filled and distended the capsular space and then flowed on into the proximal tubule which might or might not reach the kidney surface. As soon as a millimeter or so of the proximal tubule had been filled with oil, the oil remaining in the capsular space was evacuated into the pipette and the collection of glomerular fluid commenced against a slight positive pressure in the collecting system. The oil served the double purpose of identifying the punctured object as a glomerulus and, by its continued presence in the proximal tubule, gave assurance that the collected fluid was derived from the glomerulus rather than from any more distal portion of the nephron. The collection was continued until sufficient fluid had been secured for analysis or until it was terminated by some accident. In the 7 completed experiments the average amount of fluid obtained was 0.24 c.mm. Subsequent dissection of the nephron, by the methods to be described, proved whether or not any tubule bordering the glomerulus had been accidentally punctured during the experiment.

Two matters should be mentioned since they may detract from the significance of these experiments. Glomerular fluid, collected from amphibia, proved to be free[4] of protein (5). Of the 6 specimens of this series which

[4] The method cannot consistently detect concentrations below 0.03 per cent. The statement, in this paragraph, that a specimen contained no protein should therefore be taken to mean that it contained less than 0.03 per cent.

were similarly tested only two were negative, two contained between 0.15 and 0.20 per cent, and two contained amounts estimated at 0.80 per cent. This need not mean that the normal mammalian glomerulus excretes any considerable amount of protein, for the great majority of tubule fluid specimens and two from within 1 mm. of the glomerulus were protein-free. But it does suggest that glomerular capillaries leak protein readily even in the absence of gross damage, and that the glomeruli in question were affected either by the act of puncture or by the injection of oil. In the second place, the rate at which fluid was collected was about 30 per cent lower than would be anticipated on the basis of calculations made from creatinine clearances (6), the average figure in the 7 experiments being 0.7 c.mm. per glomerulus per hour. While the collections may have been incomplete due to leakage of fluid through tears in the glomerular capsules, these slow rates may suggest that the surface glomeruli, after puncture at any rate, were less active than those which lay deeper in the kidney substance.

The collections of glomerular fluid therefore were neither very numerous nor wholly free from objection. Fortunately the conclusions to which they led received support from the more numerous and satisfactory experiments upon proximal tubules.

Collection of fluid from proximal convolutions. As will be understood from an examination of figures 5 to 10, the vast majority of the tubule segments appearing on the kidney surface proved to be portions of the proximal convolutions. In a good preparation they were distended with fluid and presented the appearance illustrated in figure 1. Each segment looked precisely like its neighbor and it was impossible to distinguish anatomical portions of the proximal tubule from each other or from the distal tubule by simple observation. Two juxtaposed segments were not necessarily portions of the same nephron and their relationship to each other could only be shown by such an intratubular injection as is illustrated in figure 1 where india ink was used. The tubules were larger in adults than in young and increased in size after unilateral nephrectomy. Of the animals examined, they were largest in the opossum and skunk. In the guinea pig the continuous segments on the surface were rather longer than in the rat. An active circulation could be seen in the blood capillaries which bordered each segment and, on occasion, crossed over a tubule. If the circulation failed, or if the renal artery was clamped, the lumina of the tubules collapsed and their walls became more apparent; under these circumstances some tubules appeared white while others showed brownish granulations but these differences did not prove to be characteristic of any particular portion of the nephron. If the tubules were collapsed when first observed, they could be promptly distended by the intravenous injection of 1.0 cc. of a 10 per cent sucrose solution. The effect was much more marked than that pro-

duced by a similar amount of 0.9 per cent sodium chloride solution, and suggests that sucrose affects fluid reabsorption in the proximal tubule.

Any dilated surface tubule with its axis parallel to that of the pipette was selected and the point thrust into it; if the point were properly bevelled and the renal capsule not too thick the insertion could be made without tearing the tubule wall, damaging its capillaries, or penetrating into a deeper layer of tubules, though these accidents often occurred. The most perfect punctures were made in the rat by simply pressing the pipette tip against the tubule wall and allowing the small respiratory excursions to tease a hole for it. With the pipette inside the tubule lumen a short column of red oil was injected; this demonstrated the proper position of the pipette and, if it subsequently moved along the tubule into further convolutions, proved that there was a flow of fluid down the tubule. When the oil column had taken up a position distal to the site of puncture, the collection of fluid was commenced, the pressure in the collecting system being so adjusted as to immobilize the oil. The immobility of the oil column provided assurance that all of the fluid descending the tubule was being collected and that there was no contamination by fluid which had passed distal to it. In a few experiments metallic mercury has been substituted for the oil; this provides a more certain block but has been impossible to inject with consistent success.

There can be no doubt that fluid entering the pipette originated from within a nephron, for no appreciable amount of fluid can be collected from the normal kidney surface or from the kidney substance unless the pipette be thrust into a tubule lumen or damage a blood vessel. We have seen no indication of large currents of interstitial fluid (7) and there are no obvious spaces on the kidney surface unoccupied by tubules or blood vessels.

A number of factors combined to make the collections less simple than this description perhaps suggests. In the first place, the mere fact that the tubule appeared distended with fluid did not necessarily mean that there was an active flow within it, for frequently the injected oil would move onwards sluggishly or not at all; under these circumstances other tubules were punctured until one was found from which fluid could be collected. A fluid collection, once begun, continued steadily unless some extraneous factor intervened. In this observation and in the observation that pulsatile movements at the proximal end of the oil column, apparently transmitted to it from the glomerulus, continued without interruption, we find arguments against the existence of intermittent glomerular activity in our preparations. A second difficulty lay in the very high intratubular pressure which was particularly prominent during the infusion of hypertonic sucrose solutions. Under these circumstances the injected oil column, which at first had started down the tubule, would rush suddenly and violently back towards the pipette and emerge on the kidney surface. We

attributed this to a leak at the point of puncture and the consequent collapse of the punctured nephron by its distended neighbors. When saline infusions were substituted for sucrose, or all infusions omitted, the difficulty was encountered less often. A third difficulty lay in the tendency of the pipette point to become obstructed by some particle within the lumen or by contact with the tubule wall. When this occurred, the oncoming fluid either pushed the oil column on down the tubule or emerged on the kidney surface; in the latter event it often came with sufficient force to detach the capsule and form a visible drop of fluid around the point of puncture. The collection could be continued if the pipette were withdrawn from the lumen and the fluid picked up as it emerged from the tubule and lay beneath the oil which covered the kidney surface. Fourteen per cent of our collections have been made in this fashion. We deprecate the technique, for there might have been an admixture with the thin layer of surface fluid which may cover the kidney, but two points minimize the dangers introduced by this fluid. It was only present in considerable amounts when the renal capsule had been removed and in the immediate vicinity of the points upon which pressure was being exerted. It was readily distinguished from tubule fluid since it contained about 1.0 per cent protein, and reducing substances and chloride in the concentrations anticipated in an ultrafiltrate of blood plasma.

We have described the technique employed in the collection of fluid from any dilated surface tubule, chosen at random. Almost invariably these tubules proved to be proximal convolutions and in the middle third of this segment. The last third dips deeply into the kidney substance to join the loop of Henle, and is inaccessible by our technique (figs. 5–10). The first third appears occasionally on the surface and a special procedure was devised to identify it. A pipette containing air below the mercury and, at its extreme tip, a little oil, was thrust into a surface tubule. After the oil was injected and allowed to flow distal to the punctured point, a column of air was forced into the tubule. The air, prevented from going distally by the oil column, distended and outlined the coils of tubules on the glomerular side of the puncture. When the proximal end of this air column happened to be in a surface tubule this point was selected as the site of collection, with the assurance that it was on the proximal side of the original segment by the length of the air column. The technique was demanding but it provided a number of collections from the first third of the proximal tubule. It had the disadvantage that the sudden distension with air appeared to damage the tubule wall; during the subsequent experiment this portion of the tubule often looked white and some cellular detritus was found in the distal tubule at the time of its dissection. We have not felt that this damage disqualified the experiment, for the collection was made proximal to the region which had been distended. No evidence of similar damage from the

simple act of puncture or the injection of oil has been seen, other than the local tear in the tubule wall at the point of penetration. The glomerulus and the portion of the tubule proximal to the site of collection remained completely untouched and usually well below the kidney surface.

These methods for the collection of fluid from proximal tubules have proved quite satisfactory, especially in the rat. The distal tubules have presented particular difficulties, as yet only partially solved.

Collection of fluid from distal convolutions. Scattered over the surface of both guinea pig and rat kidneys there are occasional distal tubules which, coming to the surface, make but a single loop there and then descend again into the kidney substance (figs. 5–10). Their infrequency in comparison with proximal convolutions is indicated by the fact that only 3 out of 92 tubules, punctured at random, proved to be distal segments. Some special method of identification had to be designed for, though their diameter is smaller than that of proximal convolutions in a ratio of 2:3 when the measurements are made after death in dissected specimens (table 1), this difference is either absent or indistinguishable during life when the tubule lumina are dilated. We attempted to utilize the preferential vital staining of proximal convolutions by trypan blue in several series of experiments on guinea pigs and rats, hoping that the distal segments would appear as unstained tubules against a blue background. The attempts were unsuccessful for, though differentially stained, the contrast between the two segments could not be seen in the living animal.

It has, however, proved possible to identify distal tubules by the use of phenolsulfonephthalein. Six milligrams were injected intravenously and, 5 minutes later, the ureter was clamped to produce a maximum concentration of the dye. Under these circumstances the lumina of scattered tubules throughout the field became deeply colored and this color was particularly well seen when the tubule, ascending directly to the surface, allowed one to observe a column of fluid in depth. These colored tubules have uniformly proven to belong to distal convolutions. One such tubule was selected, its position relative to its surroundings noted, the clamp removed from the ureter, and puncture subsequently performed. Identification by this method involving, as it did, coloration of the tubule fluid made certain analyses impossible. The collection of satisfactory specimens has also been complicated by the small amounts of fluid available and the difficulty of establishing an adequate block. In 3 successful experiments the average volume collected has been only 0.07 c.mm. and its rate of collection 0.31 c.mm. per hour. While such small volumes were anticipated on theoretical grounds and were consistent with the demonstration of fluid reabsorption in the proximal tubule (6) they have imposed added difficulty on our analytical technique. When an oil column was injected into a distal tubule it would move onward in the direction of a collecting duct but then,

as collection of fluid was started, would return to the pipette point and often take up a position proximal to it. Under these circumstances of course the collected fluid was derived from distal to the point of puncture and the results of its analysis became meaningless. This difficulty was presumably associated with the low intratubular pressure and the extremely slow rate of flow which exists within the distal convolutions. Our methods for identification of distal tubules and for the collection of fluid from them are, then, still imperfect.

Methods of identifying the site of puncture. The collection and analysis of fluid possessed little or no significance unless the site of the collection could be accurately determined. The problem presented difficulties. In the earlier stages of the investigation attempts were made by our former colleague, Prof. Rudolf Kempton of Vassar College, to identify the punctured tubule by examining serial sections of a block of kidney tissue which contained the nephron in question. The method was very time-consuming and rarely provided more information than that the puncture was in a proximal or distal segment. Recourse was therefore had to the technique of maceration and dissection.

At the conclusion of each experiment, a pipette containing a 1:10 dilution of "soluble" india ink was reinserted into the tubule from which fluid had been collected, and a small quantity of ink injected. The kidney was then placed in 10 per cent formaldehyde. After two or three days of fixation it was removed and the small ink spot lying within the tubule and beneath the capsule around the puncture wound was located with a hand lens. A wedge shaped segment of kidney extending well into the papilla and containing the punctured nephron was then excised, care being taken to leave about 5 mm. of tissue around the central-lying ink mark. This block was placed in a stender dish containing concentrated hydrochloric acid and allowed to macerate until sufficiently softened for dissection. The length of the time required depends on the room temperature and the specimen therefore must be carefully watched, for a few hours' excessive maceration will destroy the structure of the nephrons completely. Under the conditions of our laboratory from one to three days was found to be most favorable. The acid was then poured from the softened tissue and it was rinsed by decanting with several changes of distilled water.

Dissection and isolation of the nephron was done under water beneath the binocular microscope at a magnification from 20 to 60 times, in the same dish which had contained the specimen from the beginning of the maceration. Strong direct light against a black background was found most useful during dissection, though transmitted light proved helpful in locating the ink within the tubule. Steel needles, frequently cleaned with an emery cushion to avoid the stickiness of the softened tissue, were used to untangle the marked nephron. No description of this procedure is possible, but its

progress is shown in figure 2. The most hazardous part of these manipulations was the final separation and disentanglement, without breakage, of

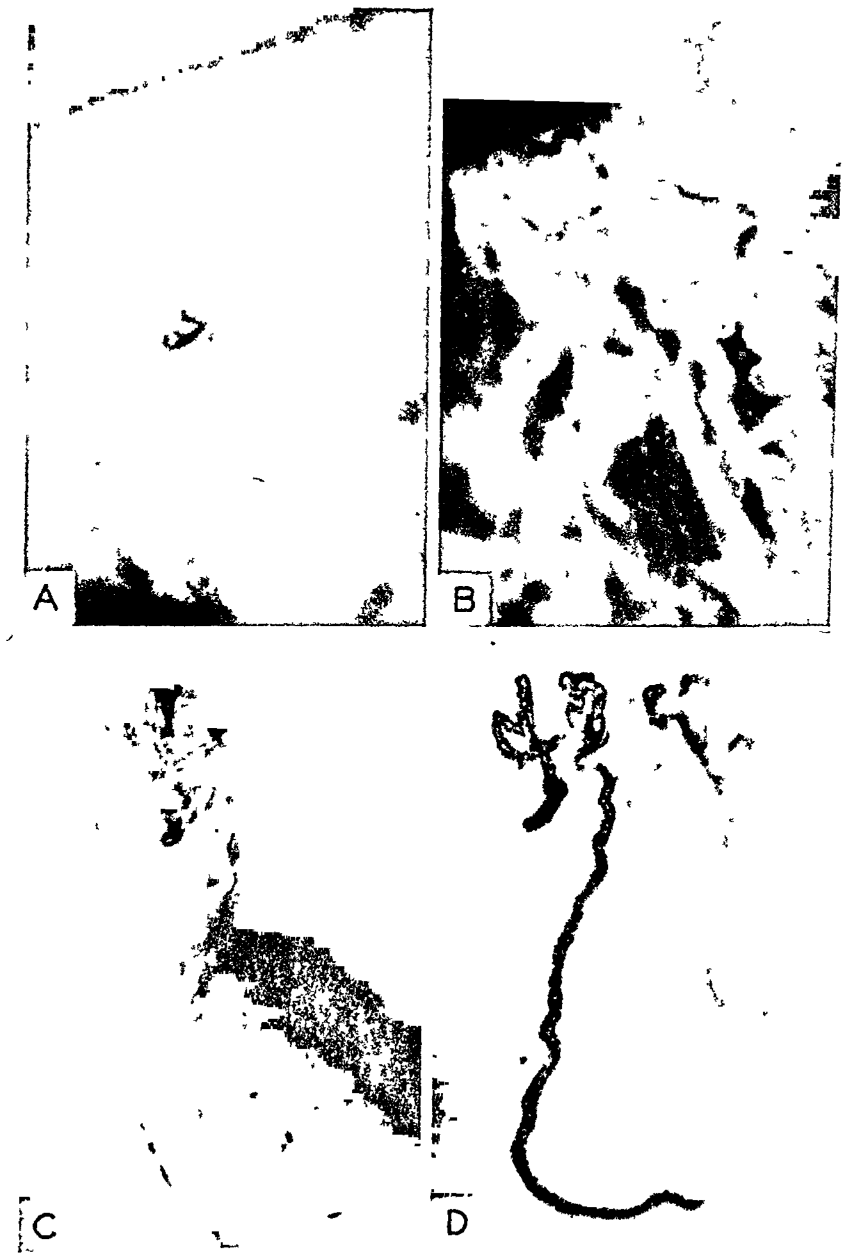
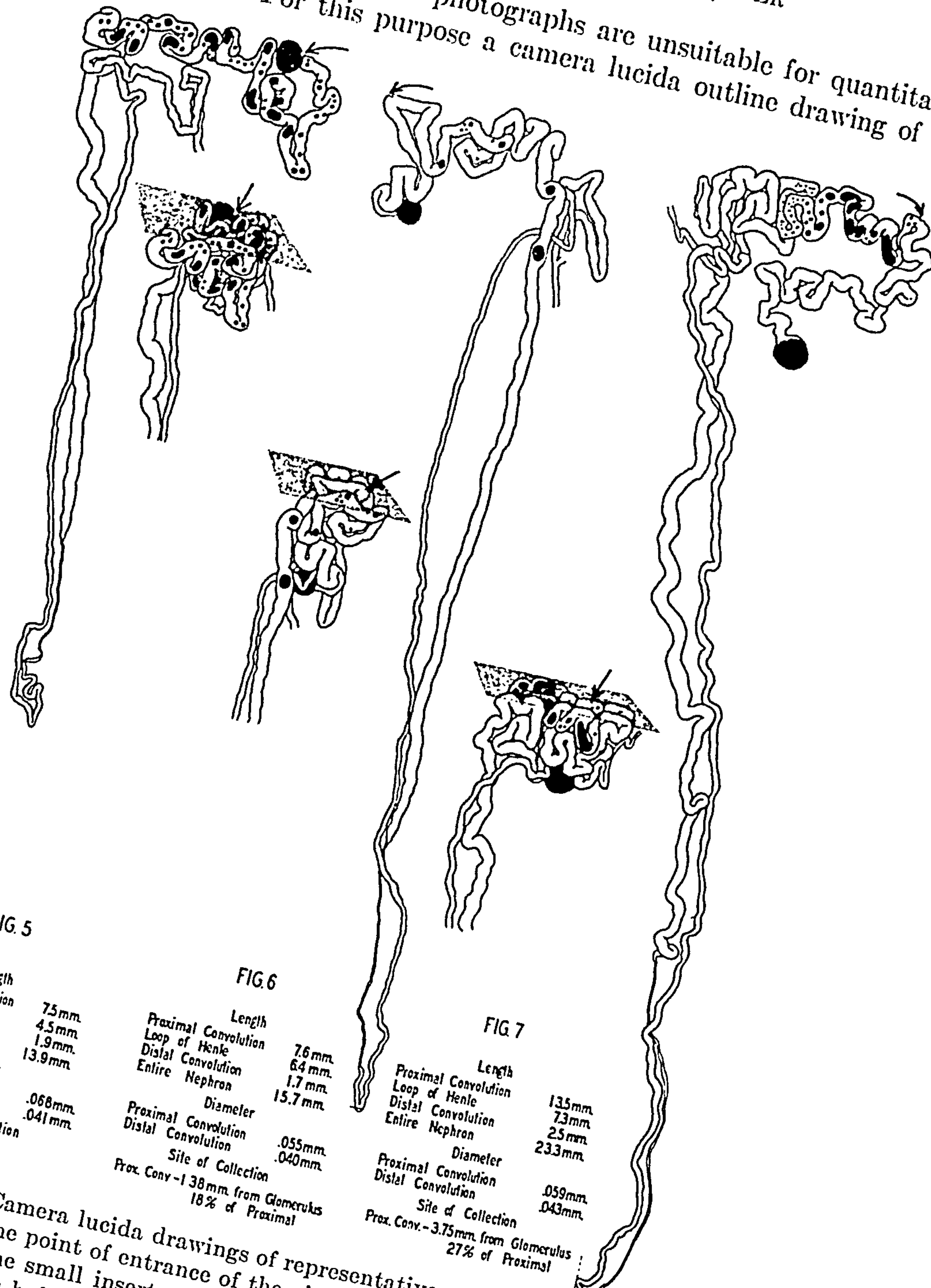


Fig. 2. Progress of dissection of a nephron (expt. 41 (6)). A, the surface of the kidney of a guinea pig after maceration showing ink in lumen of tubule. B, preliminary separation of cortical tubules; loops of proximal convolution containing ink are clearly seen. C, the isolated complete nephron showing in D, the final separation of the distal from the proximal convolution. Magnification A, C and D 22×; B, 32×.

the distal convolution from the redundant coils of the surrounding proximal convolution (fig. 2d).

a floating specimen, so that photographs are unsuitable for quantitative measurement. For this purpose a camera lucida outline drawing of the



Figs. 5, 6, 7. Camera lucida drawings of representative nephrons after microdissection showing the point of entrance of the pipette (arrow) and oil droplets in the tubule lumen. The small inserts show the position occupied by the loops of the convoluted tubules before microdissection. Figure 5, guinea pig; figure 6, guinea pig, experiment 13 (6); figure 7, rat, experiment 9 (6). Magnification 23×.

nephron at a magnification of 80 times was made, showing the point of pipette entrance and the site of oil and ink (figs. 5–10). In its preparation

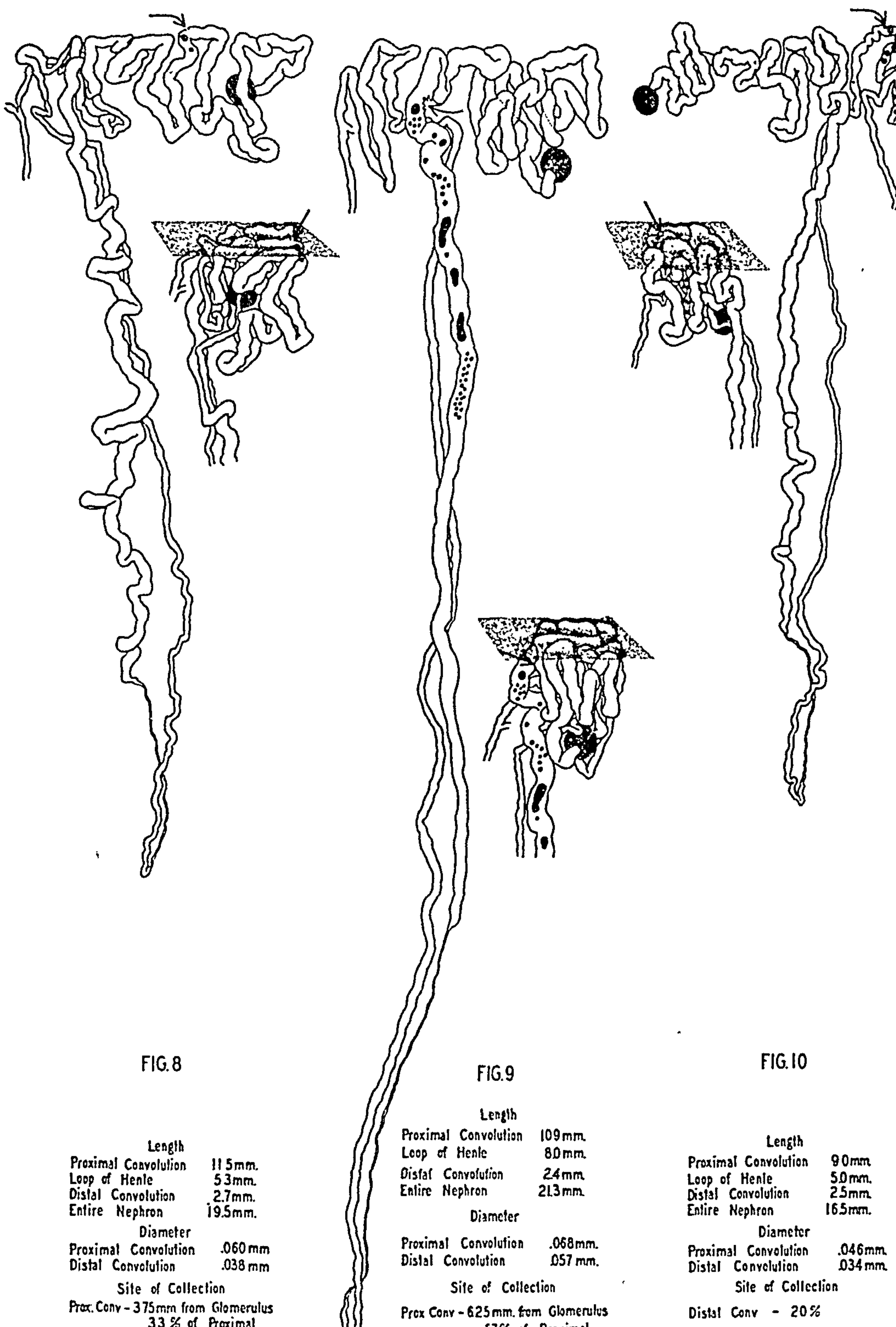


FIG. 8

Length
Proximal Convolution 11.5mm.
Loop of Henle 5.3mm.
Distal Convolution 2.7mm.
Entire Nephron 19.5mm.
Diameter
Proximal Convolution .060 mm
Distal Convolution .038 mm
Site of Collection
Prox. Conv - 3.75mm from Glomerulus
33 % of Proximal

FIG. 9

Length
Proximal Convolution 10.9mm.
Loop of Henle 8.0mm.
Distal Convolution 2.4mm.
Entire Nephron 21.3mm.
Diameter
Proximal Convolution .068mm.
Distal Convolution .057 mm.
Site of Collection
Prox Conv - 6.25mm. from Glomerulus
57% of Proximal

FIG. 10

Length
Proximal Convolution 9.0mm.
Loop of Henle 5.0mm.
Distal Convolution 2.5mm.
Entire Nephron 16.5mm.
Diameter
Proximal Convolution .046mm.
Distal Convolution .034mm.
Site of Collection
Distal Conv - 20%

Figs. 8, 9, 10. Camera lucida drawings of representative nephrons after microdissection showing the point of entrance of the pipette (arrow) and oil droplets in the tubule lumen. The small inserts show the position occupied by the loops of the convoluted tubules before microdissection. Figure 8, rat; figure 9, guinea pig, experiment 55 (6) (cf. fig. 3); figure 10, rat, experiment 58 (6). Magnification 23×.

a source of error in measurement, namely, the effect of foreshortening produced by the coiling of the loops of the convoluted tubules, was removed by placing thin glass capillary tubes across the floating tubule so that its loops were flattened down against the bottom of the dish in a single plane.

The actual measurement of the length of the various portions of the nephron was made on this drawing by means of a map measure with a small wheel with which the straightened and flattened twistings of the tubule could be easily followed. Measurements of the diameter of the tubule in various segments were made on the actual specimen at a magnification of 43 times with a filar micrometer. The mean of at least ten measurements was used as the final expression of the diameter, a value sufficiently accurate for the purpose of these experiments, though it must be recognized that such an evaluation ignores the fact that in the hypertrophied proximal convolution the straight terminal portion, comprising

TABLE 1

Measurements of hypertrophied nephrons

	PROXIMAL CONVOLUTION		LOOP OF HENLE	DISTAL CONVOLUTION	
	Length	Diameter	Length	Length	Diameter
	mm.	*mm.*	*mm.*	*mm.*	*mm.*
Rats (37)..........	11.4 (6.8–19.3)	0.058 (.04 –.080)	6.7 (5.0–9.8)	2.4 (1.2–3.0)	0.040 (.032–.050)
Pigs (37)..........	8.0 (5.5–11.6)	0.056 (.039–.079)	5.7 (3.8–8.5)	1.7 (0.9–2.8)	0.044 (.034–.062)

about one-third of its length, may be twenty-five per cent thicker than the mean diameter of the convolution as a whole. A summary of these measurements is shown in table 1.

Besides the measurement of the length of the various portions of the nephron, the distance of the point of entrance of the pipette from a glomerulus was also recorded by means of the map measure.

This completed the morphological data on the nephron. The final procedure, accomplished in part during the course of the earlier dissection, was the isolation and inspection of neighboring nephrons to make certain that they had not been entered by the pipette or torn so that a source of contaminating tubule fluid had been produced. Ink granules which had leaked from the punctured tubule lumen were found not uncommonly in the interstitial tissue and on neighboring tubules, but its presence on the surface of an intact tubule rather than in its wall or its lumen allowed an easy decision as to whether a possible contamination could have occurred. In five instances such accidents were found to have happened; tears were

seen in contiguous tubules or their lumens contained either ink or, more frequently, oil droplets which showed plainly that more than one nephron had been involved in the experimental puncture.

To sum up the advantages of the morphological procedure it may therefore be stated that an objective and permanent record which allowed accurate quantitative measurement was obtained; that the position of the point of entrance of the pipette into the tubule lumen relative to the remainder of the nephron could be accurately determined; that the adequacy of the oil block could be demonstrated and that errors due to entrance into more than one nephron, errors not appreciated during the course of the experiment, could be positively eliminated.

SUMMARY

Methods have been developed for observing the kidney surface in anesthetized mammals and for collecting fluid from single glomeruli, proximal tubules, and distal tubules. Sufficient amounts of fluid can be collected for quantitative analysis by ultramicro methods and the precise *locus* of the collection can be determined.

It is unnecessary, but a pleasure to us, to remark that these experiments are a direct and logical continuation of those initiated by Prof. A. N. Richards many years ago and to record his continuous encouragement and advice in their execution.

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THE COLLECTION AND ANALYSIS OF FLUID FROM SINGLE NEPHRONS OF THE MAMMALIAN KIDNEY[1]

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Methods have been developed for the collection of fluid from single glomeruli and tubules of the mammalian kidney (1). The amounts of fluid which can thus be collected, though small (from 0.03 to 0.70 c.mm.), were sufficient for quantitative analysis by ultramicro methods (2), and such analyses have been performed in a series of 92 experiments upon rats, guinea pigs and opossums. It was the design of the experiments to learn some of the changes which occur as fluid descends a tubule from the glomerulus toward the ureter and to establish the site of these changes, the same type of experimentation which has yielded considerable information in the amphibian kidney (3). In the present instance it has confirmed existing ideas of glomerular function, established the site of glucose reabsorption, and shed some light on the extent and type of fluid reabsorption which occurs in the proximal tubule.

Methods of analysis. The analytical methods, excepting that for sodium, were devised in connection with the experiments upon amphibia and are fully described in the publications to which reference will be made. They were carried out in small glass capillary tubes. Changes in the original techniques and details pertinent to the present investigation are summarized in the following paragraphs.

Protein (2). A polished black surface was used as the background, and a micro lamp with 300 watt bulb and spherical condenser as the source of illumination, in examining for the presence of precipitate. The concentrations of protein ascribed to glomerular and tubule fluid in the text are based on control experiments in which various dilutions of guinea pig blood plasma, assumed to contain 7 per cent protein, were used. Concentrations less than 0.03 per cent could not be distinguished with certainty. The results are only quantitative in the sense that, for example, 0.05

[1] The expenses of this work have been defrayed in large part from a grant by the Commonwealth Fund of New York. The results of the experiments were reported before the American Physiological Society at Chicago in April 1941 (This Journal **133**: 480, 1941).

per cent could be readily distinguished from 0.10 per cent. The method of Shevky and Stafford (4) was used for the macro analyses of protein in bladder urine.

Reducing substances[2] (5). The accuracy of the ultramicro colorimetric method was re-examined by comparing its results with those obtained by the Shaffer-Somogyi macro method (6) in the analysis of 6 specimens of guinea pigs' and rats' blood plasma. The results of the two methods did not differ by more than 8 mgm. per 100 cc. and the average difference was 4 mgm. per 100 cc. Fluids containing more than 125 mgm. per 100 cc. were diluted previous to analysis, since full color development in capillary tubes does not occur above this concentration and since the color becomes difficult to read accurately. Analyses of volumes of tubule fluid less than 0.10 c.mm. and of blood plasma less than 0.15 c.mm. gave incomplete color development and the results of experiments in which such volumes were used have been omitted. Heparin should not be used as an anticoagulant.

In the experiments upon guinea pigs, the concentration in tubule fluid was compared with that of two specimens of blood plasma collected at the beginning and end of the experiment, their average being obtained by interpolation to the mid-point of the experiment. Because the animals were eviscerated and a considerable interval of time separated the two plasma collections, the glucose concentration of specimen 2 averaged 40 mgm. per 100 cc. less than specimen 1. This fall in glucose concentration diminished the accuracy of the average plasma value recorded in table 1. The fall was so marked following the injection of phlorhizin, that the eviscerated guinea pig could not be used for this type of experiment unless the hepatic circulation were left intact, a procedure too difficult for routine use and employed but once (expt. 31).

In the experiments upon rats, since evisceration was unnecessary, the blood glucose was quite constant, and comparison could be made with a single specimen of plasma obtained from the animal's tail at the mid-point of the collection.

The bladder urine formed by guinea pigs during the experiments had an average concentration of reducing substances of 0.06 per cent when analyzed by the ultramicro method, and 0.13 per cent by the Shaffer-Somogyi macro method. The greater part of these substances was not glucose for 6 of the same specimens, when analyzed for fermentable sugars, contained an average of only 0.019 per cent. The values for urine have been omitted from table 1 because of the presence of these non-fermentable reducing substances but they do not, apparently, appear within the proximal tubule in sufficient concentrations to affect analyses of fluids from this site.

Creatinine (7). The procedure referred to as "method B" was used both for plasma and tubule fluid. In the preparation of protein-free filtrates of plasma we employed 1 volume of plasma, 0.5 of 10 per cent sodium tungstate, 8.5 of N/25 sulphuric acid. The accuracy of the method proved to be as previously described. The comparison between tubule fluid and blood plasma in guinea pigs was made less accurate by the rapid fall in plasma concentration (averaging 0.015 per cent) which occurred between collection of the two blood specimens; the plasma figure in table 1 was obtained by interpolation to the mid-point of the experiment. In rats the plasma concentration only changed an average of 0.003 per cent during the course of an experiment, and comparison was usually made with a single specimen obtained at the mid-point of the experiment.

Osmotic pressure (8). In previous papers the results of this measurement have been designated—somewhat inaccurately—as "total molecular concentration." The measurement consists in comparison of the vapor pressures of tubule fluid and plasma

[2] The macro analyses for reducing substances, chloride and protein were made by Miss Ethol Shiels, Department of Pharmacology, University of Pennsylvania.

by Barger's capillary method for determining molecular weight. The results given in table 1 show the number of micrometer scale divisions, each equivalent to 4.7μ, by which a column of tubule fluid changed in length during 48 hours' equilibration in a glass capillary tube against heparinized blood plasma from the same animal. In each experiment a similar comparison was made between bladder urine and plasma, and a third (control) capillary was prepared in which all of the columns were bladder urine. The results of this latter comparison, omitted from the table, show an average change in length of only 1.8 scale divisions—an insignificant difference.

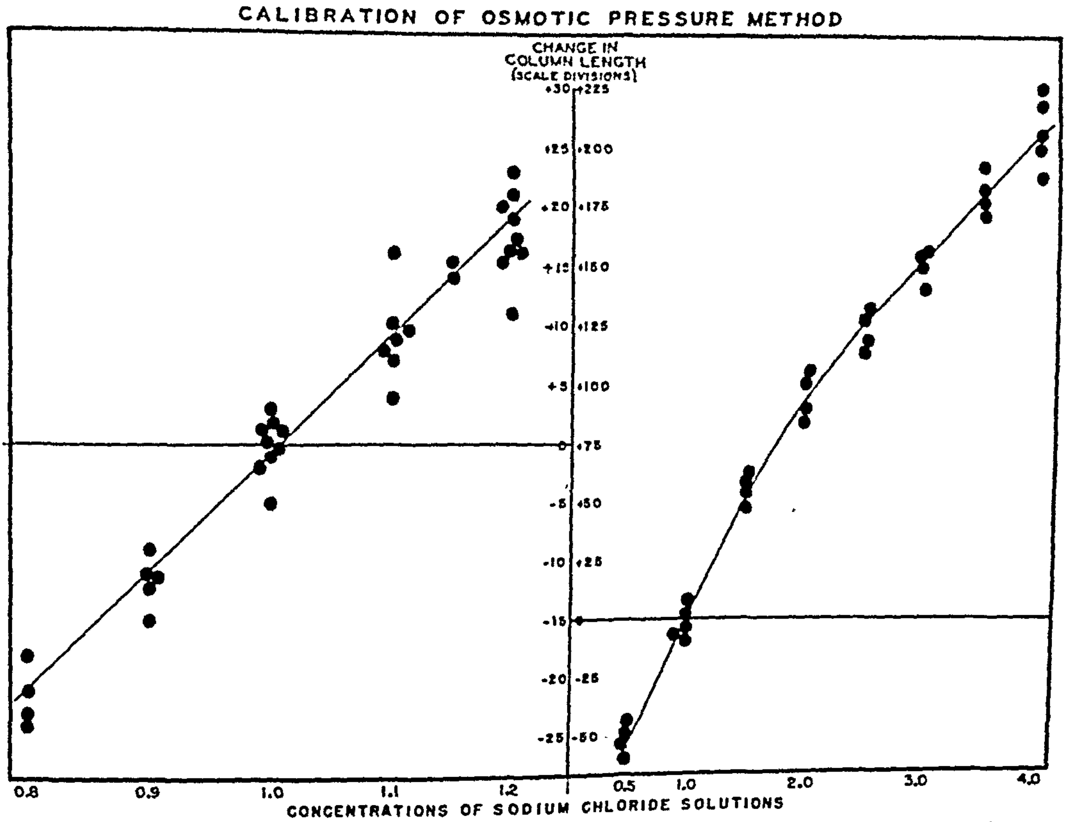

Fig. 1. Each dot represents an experiment in which blood plasma of a rat or guinea pig was compared with sodium chloride solutions of known concentrations. The ordinates represent the number of micrometer scale divisions (each 4.7μ) by which the 2 mm. column of plasma increased (+) or decreased (−) in length during 48 hour equilibration in a glass capillary tube.

In figure 3 the same results are plotted in terms of percentage differences between the fluids and plasma; this quantitative expression was obtained from a series of 11 control experiments in which columns of blood serum or heparinized blood plasma, collected from the vena cava, renal vein, or tail of normal or operated guinea pigs and rats, were equilibrated with sodium chloride solutions of known concentrations by precisely the same procedure. The results show that 10 per cent differences are readily recognizable and that the osmotic pressure of plasma is approximately that of a 1.0 per cent NaCl solution. They yield the curves shown in figure 1, reference to which enables the experimental results to be expressed as percentage differences, and they are so charted in figure 3.

In the experiments it was often necessary to use less than 0.2 c.mm. of tubule fluid; when this was the case similarly small amounts of bladder urine were used. When the difference in osmotic pressure between the two fluids being compared is less than 20 per cent, the resulting change in column length was found to be independent of the volume used; when the difference exceeds 20 per cent, the use of small volumes resulted in an osmotic pressure reading below the actual value. The great majority of the tubule fluid determinations are therefore not open to objection on this score but the osmotic pressure of the bladder urine specimens has been underestimated.

Chloride (9). The accuracy of the ultramicro method was re-examined in a series of 10 experiments on sodium chloride solutions, blood plasma and urine specimens. Each specimen was analyzed by both ultramicro and Eisenman (10) macro methods; the average difference between results by the two methods was 2.7 per cent, the maximum 4.9 per cent. The concentration in tubule fluid was usually compared with that of a single specimen of oxalated blood plasma obtained in mid-collection or at the end of the experiment. Such comparisons are justifiable for, in 2 experiments, blood specimens collected at one hour intervals showed a maximum difference of 1.5 per cent. Results are recorded in terms of milligrams of sodium chloride per 100 cc. and have been corrected for dissolved chromate and creatinine, the latter correction being only necessary in some experiments and then only in the case of bladder urine. The values reported for tubule fluid proved to be unaffected by preliminary precipitation with zinc hydroxide. Blood plasma collected from the renal vein had the same concentration as that collected elsewhere.

Sodium. An ultramicro colorimetric modification of the Butler-Tuthill (11) gravimetric method has been developed by Doctor Bott and will be described in a separate publication. Its accuracy is similar to that of the other methods employed in this investigation.

Results. The results of analyses upon 59 of the 92 specimens which have been collected appear in table 1 and figures 2 and 3. Thirty-three experiments have been excluded on the following grounds: site of collection could not be identified (eleven) or could only be identified as "a proximal convolution" (seven); no precautions ("block") against contamination of the collected fluid by fluid originating distal to the site of collection (six); more than one tubule punctured (five); inadequate fluid for accurate analysis (three); one additional experiment (B-46) will be mentioned in the text. The remaining 59 experiments have been carefully reviewed and are regarded as free from known technical fault; in each the block was adequate, the site of collection accurately determined,[3] and the analysis acceptable. In the entire series of experiments, the average duration of the collection was 21 minutes and the average amount of fluid available for one or more analyses 0.27 c.mm.

We have adhered to the practice, employed in the amphibian experiments, of defining the site of collection in terms of fractions of total tubule length rather than in terms of millimeters from the glomerulus. The experiments in table 1 have been arranged in that order and the symbols in

[3] Two experiments, 7 and 12, have been included though two units were found punctured at the time of dissection since it did not appear to us that the result of either experiment could have been influenced by this accident.

TABLE 1

Results of analyses of glomerular and tubule fluid

EXPERIMENT NUMBER	MAL	COLLECTION			GLUCOSE (NORMAL)			GLUCOSE (PHLORHIZIN)				CREATININE				CHLORIDE				OSMOTIC PRESSURE	
		Site*	Am't.	Rate	Fl.	Pl.	Fl/Pl.	Fl.	Pl.	Fl/Pl.	BU/Pl.	Fl.	Pl.	Fl/Pl.	BU/Pl.	Fl.	Pl.	Fl/Pl.	BU/Pl.	Fl. vs Pl.	BU vs Pl.
			c.mm.	*c.mm./hr.*	*mgm./100 cc.*			*mgm./100 cc.*				*mgm./100 cc.*				*mgm. NaCl/100 cc.*				*scale divisions†*	
1	G. pig	Glom.	0.40	0.50	96	104	0.92													−1	+137
2	G. pig	Glom.	0.45	0.71	88	92	0.96													−2	+83
3	G. pig	Glom.	0.18	0.64	175	177	0.99														
4	G. pig	Glom.	0.19	0.38	98	102	0.96														
5	G. pig	Glom.	0.06	—								65	55	1.18	87.3						
6	G. pig	Glom.	0.24	0.66								34	33	1.03	125.7						
7	G. pig	Glom.	0.15	1.12								29	30	0.97	151.6						
		Proximal																			
8	G. pig	0.3/ 7.1	0.37	1.10	115	140	0.82													−4	+108
9	Rat	0.3/ 6.8	0.44	0.98								22	20	1.10	167.8					−6	+238
10	G. pig	1.0/ 9.8	0.06	0.40												810	647	1.27	0.51		
11	Opossum	0.7/—	0.27	1.35	65	75	0.87													+4	+108
12	Rat	1.5/12.9	0.66	3.30	170	193	0.88									700	652	1.07	0.23		
13	G. pig	1.4/ 7.6	0.26	0.98								33	28	1.18	30.6						
14	Rat	2.1/10.8	0.23	0.92	65	86	0.76									837	647	1.30	0.21		
15	Rat	2.5/11.8	0.08	0.60																−1	+193
16	G. pig	1.5/ 6.8	0.21	—	47	121	0.39														
17	G. pig	1.6/ 7.4	0.35	—	68	213	0.32													−15	+41
18	Rat	3.1/12.9	0.24	1.03				190	123	1.54	50.0					910	626	1.45	0.26		
19	Rat	3.8/13.5	0.11	—				242	179	1.35	20.4										
20	G. pig	3.1/11.2	0.30	0.67	31	92	0.34													+1	+18
21	G. pig	2.5/ 9.1	0.51	0.87	110	114	0.97													−3	+170
22	Rat	4.3/14.3	0.08	0.32																−2	+76
23	Rat	4.0/13.5	0.15	0.47								55	40	1.38	86.5	770	637	1.21	0.20		
24	Rat	3.3/10.8	0.63	1.57	80	198	0.40					49	25	1.96	—	940	—	—	—		
25	Rat	2.9/ 9.3	0.21	1.05												870	647	1.34	0.67		
26	Rat	6.5/19.3	0.58	1.51	48	171	0.28									986	677	1.46	1.08		
27	G. pig	1.9/ 6.0	0.34	1.02								35	28	1.25	37.9						
28	G. pig	2.9/ 9.1	0.13	0.52	52	93	0.56														

29	Rat	3.9/10.8	0.10	—												916	636	1.44	0.68	−6	−8
30	G. pig	3.1/ 8.3	0.51	1.53	38	59	0.64														+115
31	G. pig	3.1/ 8.3	0.12	0.36				390	256	1.52	8.1										
32	Rat	6.8/17.5	0.19	0.88				321	193	1.66	—	51	29	1.76	47.4						
33	G. pig	2.1/ 5.5	0.40	0.50	68	152	0.45													0	+58
34	G. pig	3.4/ 8.5	0.25	—	45	97	0.47													−7	+137
35	Rat	3.6/ 9.0	0.48	1.60								51	24	2.12	50.8					−2	+161
36	Rat	5.5/13.1	0.70	1.68				144	67	2.15	63.5	54	26	2.08	58.3	940	647	1.45	0.19		
37	Rat	4.8/11.0	0.20	0.75	25	144	0.17														
38	Rat	3.8/ 8.8	0.20	0.80				164	66	2.48	—	84	39	2.16	40.6					−50	+49
39	Rat	2.6/ 6.0	0.42	1.01								77	36	2.14	38.2						
40	Rat	5.5/12.9	0.07	—				270	144	1.88	52.6										
41	G. pig	2.9/ 6.8	0.08	0.22								63	44	1.46	23.8						
42	Rat	3.9/ 8.5	0.14	0.70								63	34	1.85	125.0						
43	Rat	4.7/10.5	0.31	0.62				440	147	3.00	40.0					805	597	1.35	0.15	−6	+139
44	Rat	3.5/ 7.5	0.06	1.20								34	21	1.64	—						
45	Rat	5.3/11.0	0.06	—				282	113	2.74	29.3										
46	G. pig	4.3/ 9.0	0.17	0.54												930	644	1.45	1.04		
47	Rat	4.4/ 8.8	0.09	0.26												866	596	1.45	0.17		
48	G. pig	4.0/ 8.0	0.17	0.34								82	44	1.87	134.6						
49	Rat	5.8/11.5	0.22	0.78				234	91	2.53	39.4									−6	+112
50	Rat	5.5/10.8	0.10	0.60				220	86	2.56	54.4										
51	Rat	5.5/10.5	0.05	1.00																+2	+221
52	G. pig	2.9/ 5.5	0.32	0.64								53	36	1.47	41.0						
53	G. pig	6.4/11.2	0.41	1.12	32	126	0.25														+178
54	Rat	8.0/14.0	0.45	0.60				280	118	2.38	59.6					900	652	1.38	0.22	−3	+65
55	G. pig	6.3/10.9	0.31	1.10	18	118	0.15													−2	+145
56	G. pig	7.7/10.0	0.24	0.39	22	91	0.24													−3	+111
		Distal																			
57	Rat	0.5/ 3.5	0.05	0.48																−24	+144
58	Rat	0.5/ 2.5	0.03	0.06																−7	+104
59	Rat	0.5/ 2.0	0.12	0.40																−25	+65

Abbreviations: "G. pig" = guinea pig; "Glom." = glomerulus; "Fl." = glomerular or tubule fluid; "Pl." = blood plasma; "BU" = bladder urine. In columns describing glucose analyses "Phlorhizin" means that the drug has been administered to these animals before the experiment, "normal" that it has not been administered.

Sodium analyses: Tubule fluid and blood serum were analyzed for sodium in experiments 12 and 26; expressed as mgm. Na per 100 cc., the concentrations in tubule fluid were 350 and 340, in serum 334 and 334; the fluid/serum concentration ratios were therefore 1.06 and 1.02. In these serum analyses, as in those for glucose, creatinine and chloride in plasma, a correction of 7 per cent has been added so that the results may appear in terms of plasma water.

* The numerator of the fractions in this column is the measured distance, in millimeters, from the beginning of the convolution to the point of the fluid collection; the denominator is the measured total length of the convolution.

† The recorded changes are those of the central fluid column, concomitant changes in peripheral plasma columns being omitted.

figures 2 and 3 have been plotted on that basis. The figures in column 3 of the table will permit rearrangement of the data on the basis of absolute length.

Since a comparison is being made between a protein-free fluid and blood plasma, the concentrations in the latter should be reported on the basis of plasma water. A plus correction of 7 per cent has therefore been added to the results of the plasma analyses. No such correction is indicated in the case of osmotic pressure measurements (12).

Protein. Forty-one specimens of fluid from glomeruli and proximal tubules were tested for protein. No protein was found in 25 specimens; the circumstances of the test were such that 8 of these would have been positive had they contained 0.03 per cent, 17 had they contained 0.08 per cent. Of this group two were collected from glomeruli and two from within 1 mm. of a glomerulus. Sixteen specimens gave positive tests for protein; 14 of these contained less than 0.2 per cent and 9 less than 0.08 per cent. Twelve specimens of bladder urine, collected at the end of the experiments, contained an average of 0.17 per cent.

These results confirm the present belief that normal glomerular fluid contains either no protein or, at most, very small amounts. They do not distinguish between these two alternatives, but they have the virtue of excluding any third possibility. It was demonstrated that slight mechanical trauma to glomerular capillaries, far short of actual rupture, will make them permeable to gross amounts of protein.

Reducing substances. The 22 experiments summarized in table 1 and figure 2 prove that glucose occurs in glomerular fluid in concentrations similar to those in blood plasma and is reabsorbed as fluid flows through the proximal tubule. The site of glucose reabsorption is thus identical in these animals and in amphibia (16) and, as in amphibia, the process is practically completed by the first half of the proximal convolution.

The amount of glucose reabsorbed by the kidney tubules is not fixed but, like other aspects of renal function, is capable of variation. It increases, for example, during moderate rises in plasma glucose concentration as the absence of glycosuria under these circumstances indicates. Two mechanisms might be concerned in such an increase of the reabsorptive process. As plasma concentration rises, fluid reaching the latter part of the proximal convolution, normally free of glucose, might now contain it and this portion of the convolution share in the reabsorption. Alternatively, larger amounts of glucose might be reabsorbed by the earlier part of the proximal segment. In the present experiments this second alternative would seem to be demonstrable. When collections from comparable levels of the tubule are examined (table 1, expts. 16 and 17, 28 and 33) the tubule fluid/plasma ratios are seen to be very similar though the plasma glucose concentrations differ widely. Unless large and fortuitous changes in glomerular filtrate volume occurred in these nephrons, the results prove that the earlier parts

of these tubules reabsorbed more glucose when it was presented to them in increased amounts.

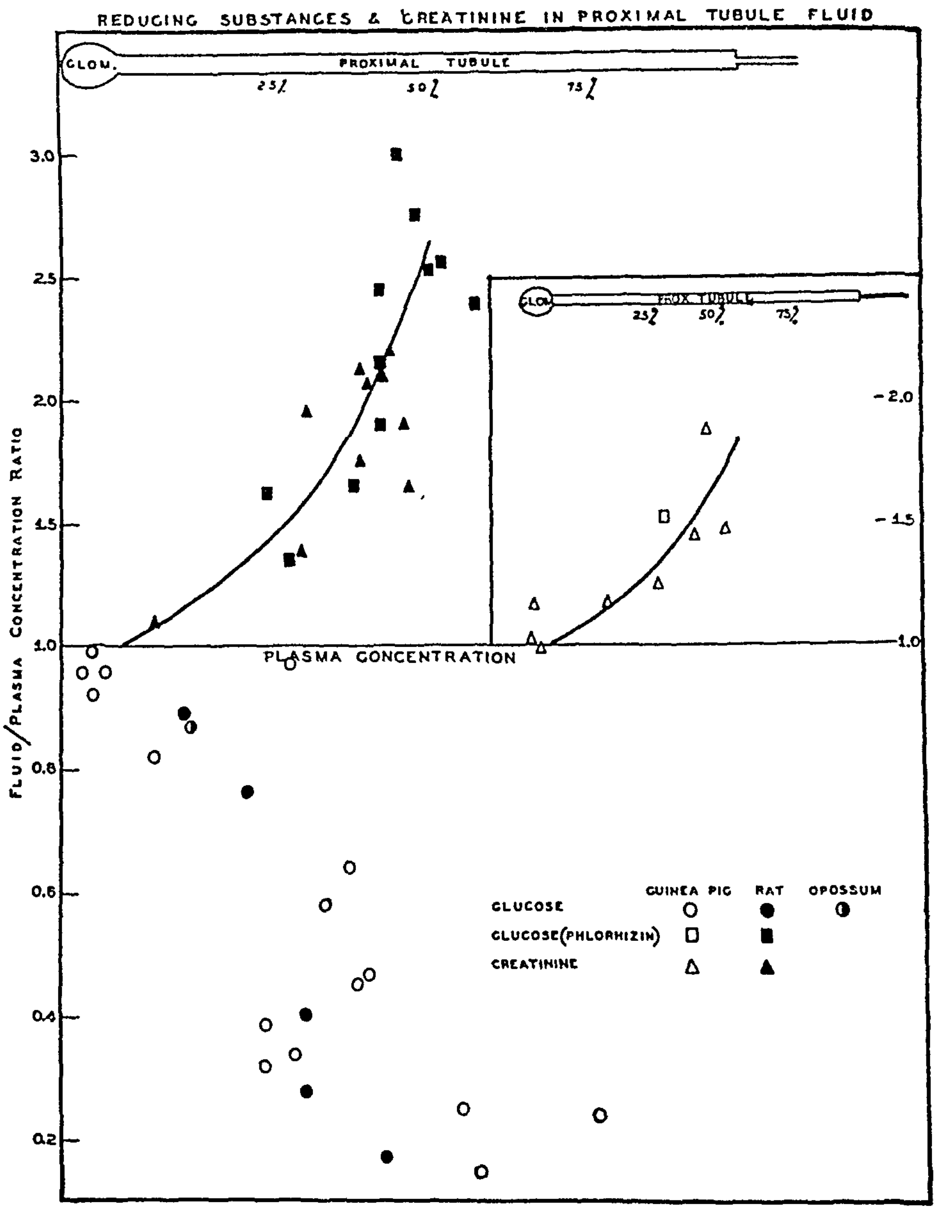

Fig. 2. Chart showing concentration ratios between blood plasma and fluid collected from glomeruli and proximal tubules with respect to exogenous creatinine, and to reducing substances with and without the preliminary injection of phlorhizin. Sites of fluid collections can be identified by reference to the diagrammatic tubules. The inset contains results of experiments upon guinea pigs, separated from those upon rats because the fluid/plasma concentration ratios were of a different order. The curves are drawn to indicate the concentration ratios which would result from the reabsorption of 12.5 per cent (inset 7.5 per cent) of the fluid of glomerular filtrate by each 10 per cent of the tubule length.

In the single experiment (table 1, expt. 24) in which creatinine analyses permitted calculation of glomerular filtrate volume in the nephron

under examination, it was computed that glucose was reabsorbed at the rate of 0.001 mgm/mm. of proximal tubule/hour.

Fluid reabsorption. In 11 experiments on rats, and 1 on a guinea pig, 200 mgm. of phlorhizin per kilogram were injected subcutaneously 30 minutes before the preparation was begun. The fluids, subsequently collected from portions of the proximal tubules, were analyzed for reducing substances and the results of these analyses appear in table 1 and figure 2. As has been believed, and demonstrated in amphibia (16), the reabsorption of glucose by the proximal tubule is arrested. If this alone occurred the concentration in tubule fluid would approximate that in blood plasma but, since it rises markedly above plasma concentration, some additional process must be involved. Only two processes could be concerned; the first, an active secretion of glucose by the proximal tubule, does not merit consideration (16); the second is the reabsorption of fluid and this we believe the experiments to demonstrate.

The problem of fluid reabsorption has also been examined by determinations of exogenous creatinine. In 18 experiments on rats and guinea pigs, following the subcutaneous injection of 300 to 500 mgm. of creatinine/kgm., fluid was collected from glomeruli and portions of the proximal tubules. The results of these analyses, and their comparison with plasma also appear in table 1 and figure 2. As was the case with glucose, the concentrations in tubule fluid rise markedly above those in plasma. In 4 experiments, both analyses were performed on the same specimens of tubule fluid with the following results:

	TUBULE FLUID/PLASMA CONCENTRATION RATIOS			
	B-42	32	36	38
Creatinine	1.55	1.76	2.15	2.16
Glucose	1.42	1.66	2.08	2.48

The similarity between the results obtained by these two methods make it unnecessary to consider the possibility of creatinine secretion.[4] The fluid/plasma ratios with this substance, as with glucose, may be attributed to the reabsorption of fluid.

[4] It could be argued that some creatinine was secreted by the normal rat but that secretion was abolished by phlorhizin in these 4 experiments. Such an effect of phlorizin has been described in man (13) but it has not been reported in other mammals and an examination of table 1 does not suggest that phlorhizin has lowered the concentration ratio of creatinine in these experiments (expts. 35 and 36, 38 and 39). Simultaneous inulin and creatinine clearances have not been performed in rats but the similarity between these two measurements in other lower mammals (14) (15) is also acceptable evidence against the secretion of creatinine.

The two series of experiments[5] may then be considered together. They demonstrate the reabsorption of very large amounts of fluid by the proximal tubules of both animals. It will be observed that the concentration ratios rise more and more abruptly as fluid is collected further and further from the glomerulus. This suggests that similar amounts of fluid are reabsorbed per unit of tubule length, for such a reabsorption would produce a progressively greater effect upon the concentration ratios as these ratios increase. The suggestion was tested by drawing the curves in figure 2 (see legend) and, since the data fit them, the suggestion may be accepted. In rats about 12.5 per cent, in guinea pigs about 7.5 per cent of the fluid of glomerular filtrate is reabsorbed by each 10 per cent of the first half of the proximal convolution.

The average concentration ratios, at a point half way down the proximal tubule, demonstrate that 60 out of each 100 cc. of glomerular fluid has been reabsorbed in rats, 40 out of each 100 in guinea pigs. It is technically impossible (1) to collect fluid from the end of the proximal convolution but it seems proper to assume that the fluid reabsorption continues, though necessarily to a lesser extent in rats, throughout the second half of this segment. The reabsorption of a total of 80 out of each 100 cc. of glomerular fluid, with a consequent concentration ratio of 4.0, would seem a conservative estimate. If this estimate be allowed, it follows that only 18 or 19 cc. of fluid need be reabsorbed by the entire loop of Henle and distal convolution to account for the concentration ratios observed in the bladder urine of these animals. This predominance of the proximal tubule in fluid reabsorption was not anticipated. It is the reverse of the situation which has been demonstrated in amphibia (16). It supports the prescient suggestion of Homer Smith (17) that "a great part of the water might be reabsorbed in the proximal tubule."

It is pertinent to inquire the effect of changes in the volume of glomerular filtrate upon this fluid reabsorption, for the question constantly arises in studying the rôle of the glomerulus in diuresis. When glomerular filtrate increases, does the tubule continue to reabsorb the same amount of fluid as previously or does it reabsorb the same percentage of the fluid now

[5] Two experiments are selected for special mention. The effect of phlorhizin in abolishing glucose reabsorption was shown particularly well in experiment B-42, omitted from table 1 because the site of collection was not accurately determined. Two collections were made from the same site in the same proximal tubule; the first, previous to the intravenous injection of phlorhizin, showed a fluid /plasma ratio of 0.48; the second, after the injection, a ratio of 1.42. Experiments 40 and 18 illustrate well the increasing concentration ratios of fluid collected at increasing distances from the glomerulus; two collections were here made from the same proximal tubule; in the first, 43 per cent of the distance from glomerulus to loop of Henle, the ratio was 1.88; in the second, 24 per cent of the same distance, it was 1.54. A drawing of this nephron appears in figure 4.

reaching it in greater amounts? In the present experiments the percentage rather than the amount of reabsorption remained constant; fluids descending the tubule at very different rates ("rates of collection") showed very similar degrees of concentration (table 1, expts. 35 and 38). This observation applies only to the site of experimentation: early proximal tubule. It does not imply that the same percentage reabsorption would persist in the presence of higher rates of glomerular filtration, but it serves as another example of the adaptability of tubule activity which was noted in connection with glucose reabsorption.

The validity of using these concentration ratios in tubule fluid to measure fluid reabsorption may be examined by inquiring what demands they make upon the rate of glomerular fluid formation. To supply the amounts of creatinine and glucose found in tubule fluid, glomeruli in guinea pigs must have formed an average of 1.00 c.mm. of fluid/hr. (7 expts.) with a maximum of 1.37, and in rats 1.96 c.mm./hr. (17 expts.) with a maximum of 3.76.[6] These demands are reasonable, for fluid has actually been collected from a guinea pig glomerulus at the rate of 1.1 c.mm./hr. and from very close to a rat glomerulus at 3.3 c.mm./hr.

It may seem that a more direct way of measuring fluid reabsorption than the use of concentration ratios would have been to compare the amounts of fluid collected from various levels of the proximal tubule. The technical difficulties involved in making these measurements, and the improbability of the assumption that glomeruli in different animals are forming fluid at similar rates, diminish the significance of this procedure. Nevertheless a general trend in the anticipated direction appears when the experiments are grouped according to the sites of collection; this trend is sufficiently definite in the experiments on rats to confirm the proof that considerable fluid is reabsorbed in the proximal convolution:

	AVERAGE RATE OF COLLECTION (CU. MM./HR.) FROM PROXIMAL CONVOLUTION		
	First fifth	Second fifth	Third fifth
Rats	1.7 (3)	1.1 (8)	0.8 (13)

Additional information as to the character of this fluid reabsorption is obtained by an examination of the osmotic pressure estimations.

[6] When the amounts of fluid formed per glomerulus per hour are calculated from the bladder urine analyses in these experiments, the average, in 17 guinea pigs, was 1.3 and in 17 rats, 2.1 c.mm. The data are imperfect for the procedure was not designed for the performance of clearance experiments, but they may be regarded as supporting evidence for the above results. Incidentally, since they are computed on the basis of all glomeruli being in constant activity, their agreement with the results from single nephrons constitutes evidence against intermittence. (See also (1, p. 569).)

Osmotic pressure. The osmotic pressure of fluid from glomeruli and the proximal tubules of rats, guinea pigs, and an opossum was directly compared with that of blood plasma in 21 experiments. The results, recorded in table 1 and figure 3, show substantial identity between the two fluids. Eight additional experiments gave similar results but are omitted because the sites of collection were not accurately determined. These results are analogous to those which have been described in the case of amphibia (8). Since the tubule contents, during and after the reabsorption of large

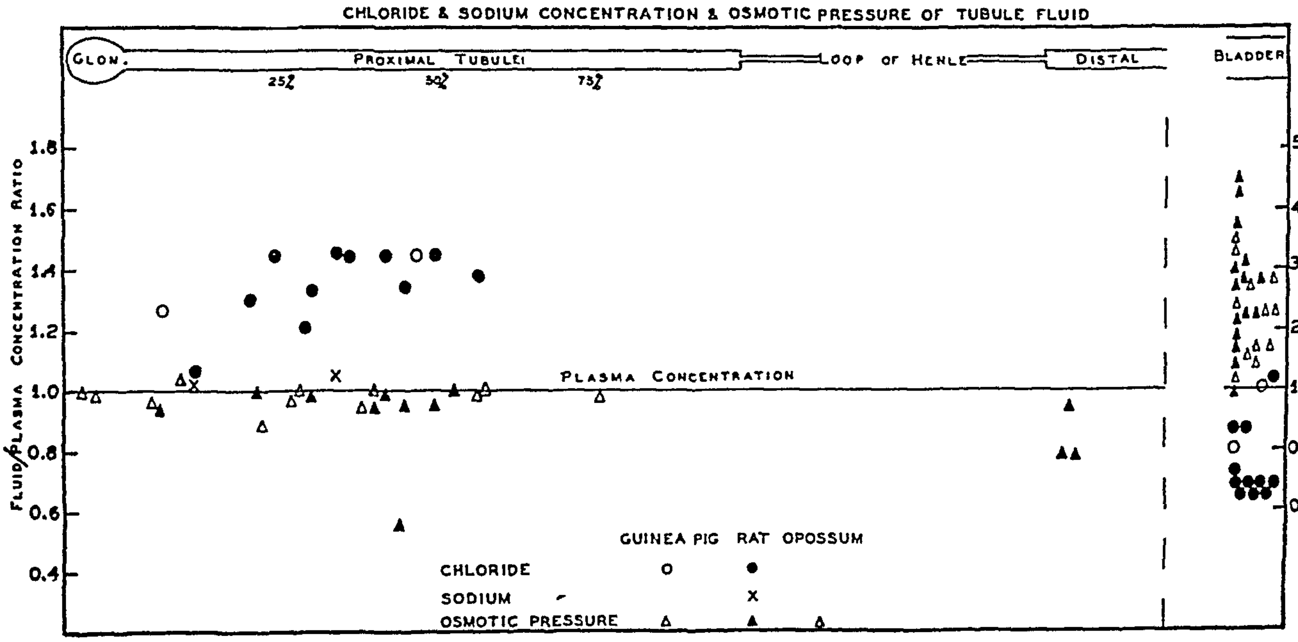

Fig. 3. Chart showing concentration ratios between blood plasma and fluid obtained from glomeruli, proximal and distal tubules and bladder with respect to osmotic pressure and concentrations of chloride and sodium. The ordinates on the left apply to glomerular and tubule fluids, those on the right to bladder urines. The values for osmotic pressure were obtained by interpolating the observed change in column length on the curves of figure 1. Site of fluid collections can be identified by reference to the diagrammatic tubule.

amounts of fluid, thus remain in osmotic equilibrium with blood plasma,[7] it must be concluded that the reabsorbed fluid is isosmotic with plasma.

Three specimens were collected from the *distal* convolutions of rats under

[7] The statement must be qualified to this extent; the fluid is certainly not hypertonic to blood plasma, but a number of the experiments suggests that it is slightly hypotonic. In all but 2 experiments the difference is scarcely beyond the error of the method but, if it be accepted as valid, indicates that these fluids have an osmotic pressure about 5 per cent less than that of plasma. In 2 experiments (17 and 39) the difference is far outside any error in the method and both tubule fluids are definitely hypotonic to blood plasma. No technical errors were recognized. It may be significant that the bladder urines in these 2 experiments have lower osmotic pressures than the great majority of such specimens.

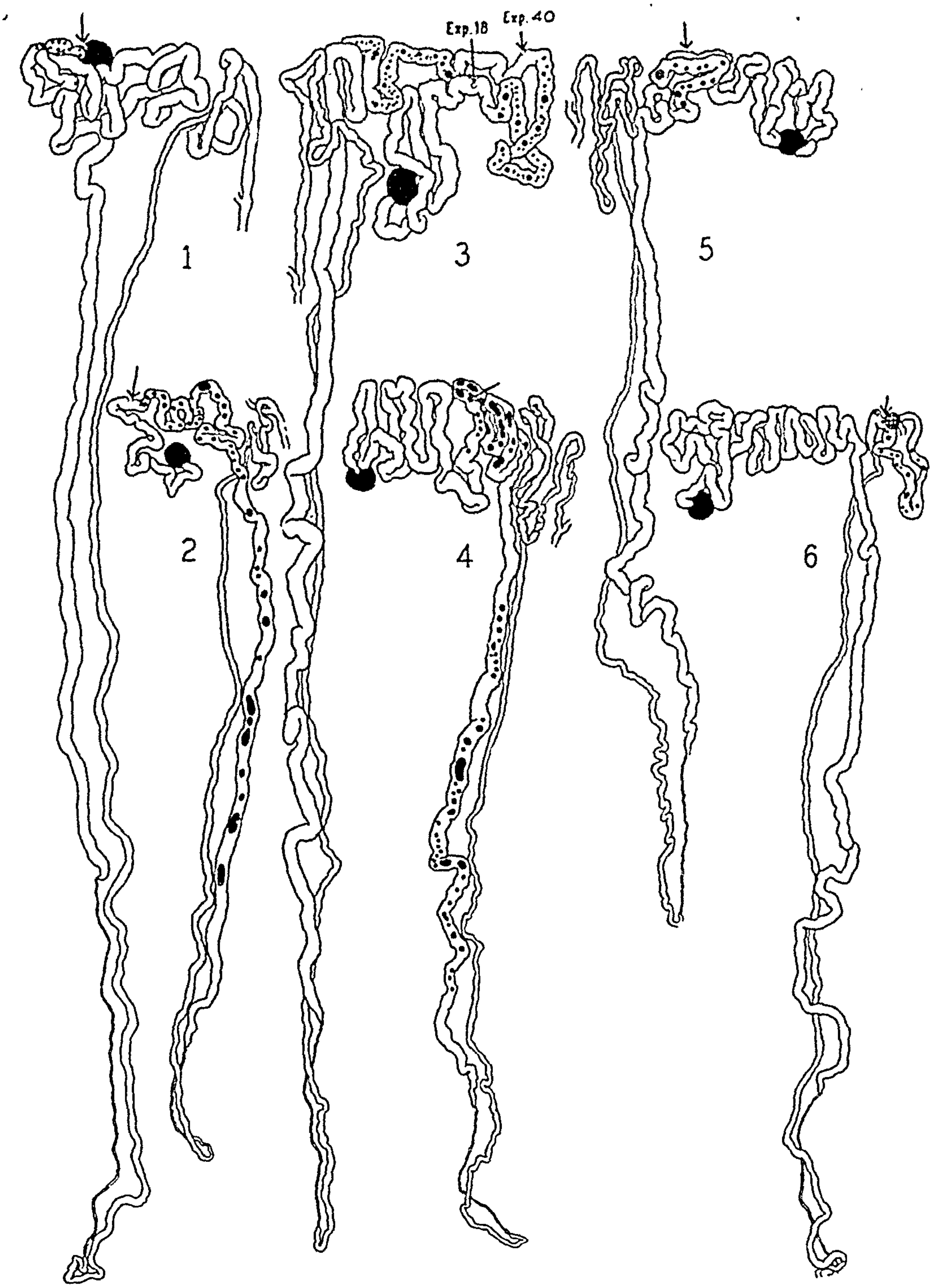

Fig. 4. Camera lucida drawings of nephrons after microdissection showing the point of entrance of the pipette and the oil and mercury blocks in the tubule lumen beyond the site of collection. The arrow shows point of entrance, solid black drops

circumstances which prevented contamination by fluid from the collecting ducts (table 1, expts. 57 to 59). Two of these specimens (and one additional specimen which was collected without this precaution) had osmotic pressures definitely below those of blood plasma, being approximately equivalent to an 0.8 per cent sodium chloride solution (fig. 1). In both cases the osmotic pressure of bladder urine was well above that of blood plasma. More data are required before any conclusions may be drawn, but if the low osmotic pressures of these tubule fluids be attributed to an active reabsorption of chloride and the high osmotic pressures of the bladder urines to a reabsorption of pure water (as distinct from fluid), then it would appear that the site of chloride reabsorption must be proximal to that of water. It has been customary to think that the site of water reabsorption, and of the increased osmotic pressure consequent upon it, lies in the loop of Henle. All three of these fluids had traversed the loop of Henle and yet did not show any increase in osmotic pressure. Two of them (expts. 57 and 59) may have traversed this loop with abnormal rapidity, but insofar as they permit a suggestion it must be that the site of water reabsorption is in the late distal tubule or even in the post-distal connecting tubule rather than in the loop of Henle.

Chloride and sodium. Since the fluid remaining in the proximal tubule is, as has been stated, in osmotic equilibrium with blood plasma, it had been anticipated that the chloride concentration of the two fluids would be the same. On the contrary, proximal tubule fluid proved to possess a distinctly higher chloride concentration than did plasma. This difference is demonstrated by the 14 experiments presented in table 1 and figure 3, and is supported by 3 additional experiments in which the site of collection was not accurately determined. It is interesting to recall that a similar difference between the chloride concentrations of proximal tubule fluid and plasma

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are oil and the cross-hatched sphere in drawings 5 and 6 is a droplet of mercury. Magnification 23×.

1. Hypertrophied guinea pig nephron from experiment 2. Site of collection, glomerular.

2. Normal guinea pig nephron from experiment 16. Site of collection 22 per cent down the proximal convolution.

3. Hypertrophied nephron from phlorhizinized rat. Two experiments, 18 and 40, were completed on the one tubule. Experiment 18, site of collection 24 per cent and experiment 40, 39 per cent down the proximal convolution.

4. Hypertrophied nephron from the phlorhinized rat of experiment 45. Site of collection 47 per cent down the proximal convolution.

5. Hypertrophied nephron from rat of experiment 35. Oil and mercury used to block the lumen. Site of collection 40 per cent down the proximal convolution.

6. Hypertrophied nephron from the rat of experiment 59. Oil and mercury used to block the lumen. Site of collection 25 per cent down the distal convolution.

was suspected in amphibia (8). There, the difference was so small in degree as to make its significance open to doubt. Here, it is altogether definite. Expressed as sodium chloride, the average concentration in tubule fluid is 0.870 per cent (0.7 to 0.986), the average in plasma 0.639 per cent (0.596 to 0.677). The fluid/plasma concentration ratio reaches an average of 1.40 in the first third of the proximal tubule and remains at that point without further increase throughout the second third of this segment. The most logical explanation[8] of these events is that chloride appears in glomerular fluid in concentrations appropriate to an ultrafiltrate of blood plasma, is concentrated in the early proximal by the reabsorption of a nearly chloride-free fluid, and is maintained at this concentration by the reabsorption in the later proximal tubule of a fluid containing approximately 1.4 times the chloride concentration of plasma.

The presence of a chloride concentration averaging 0.870 per cent in proximal tubule fluid is not, in itself, inconsistent with the recorded osmotic pressure determinations, for they indicated the fluid to be in equilibrium with blood plasma and therefore to possess a pressure close to that of a 1.0 per cent sodium chloride solution. It does, however, imply the absence of some other of the osmotically active constituents of plasma, unless the unlikely explanation of a depression of osmotic activity be considered. Ignoring this latter possibility, the most probable deficit would be in the bicarbonate ion. We have as yet no direct evidence that bicarbonate is reabsorbed and no quantitative determinations of pH have been made on tubule fluid; but the concentration of the sodium ion, in the two tubule fluids in which it was measured (table 1, expts. 12 and 26), was insufficient to cover in the one instance more than 40 per cent, in the other instance any, of the normal bicarbonate concentration of plasma in addition to the chloride demonstrably present in the tubule fluids. It may be observed that fluids of this general type, in osmotic equilibrium but electrolyte disequilibrium with blood plasma, are present elsewhere in the body, notably in the intestinal tract, eye, and central nervous system.

The majority of the bladder urine specimens in these experiments were hypotonic to blood plasma in respect to chloride. Some further portion of the nephron must therefore reverse the concentration ratio of the proximal segment and preferentially reabsorb the chloride ion.

[8] This explanation cannot be stated as a fact until the chloride concentration of glomerular fluid has been proven identical with that of plasma. This demonstration has been made in amphibia (9) but not in the present experiments. It is favored by observing that the 4 fluids with lowest concentration ratios were collected from the first third of the tubule. It is opposed by a single analysis of glomerular fluid (unlisted expt. B-46) which showed a fluid/plasma ratio of 1.40; this fluid was collected at the abnormally slow rate of 0.12 c.mm./hr. and we do not feel that its analysis deserves credence until more data are available.

SUMMARY

Occasional glomeruli, in guinea pigs, and certain portions of the proximal and distal tubules in a variety of mammals, are accessible to observation on the kidney surface during life. Fluid can be collected from these units in sufficient amounts to permit quantitative analysis and the precise site of the collection can be identified. A series of experiments are reported in which the composition of fluid thus collected has been compared with that of blood plasma in respect to protein, reducing substances before and after phlorhizin, exogenous creatinine, osmotic pressure, chloride and sodium.

The analyses indicate that glomerular fluid, entirely or nearly free of protein, contains reducing substances and creatinine in concentrations similar to those existing in plasma water. Within the proximal convolution, all of the reducing substances and at least two-thirds of the fluid are reabsorbed. This fluid reabsorption is an isosmotic process, accomplished without producing any increase in osmotic pressure of the fluid remaining within the tubule; it is not, however, a purely passive reabsorption of unchanged glomerular fluid for the chloride concentration of tubule fluid increases to a level 1.4 times that obtaining in blood plasma. The existence of this increased chloride concentration apparently requires that bicarbonate be preferentially reabsorbed by the proximal tubule, but the point has not yet been directly examined. The tentative conclusions, drawn from a small number of experiments with distal tubule fluid, are stated in the body of the paper.

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AN INVESTIGATION OF CHEMICAL TEMPERATURE REGULATION[1]

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Chemical temperature regulation may be defined as the increase in metabolic rate above the basal value when a resting and fasted animal is exposed to cold. There are two possible components of chemical temperature regulation, namely, 1, an increase of metabolism without shivering and presumably due to hormones which stimulate energy metabolism, and 2, shivering.

Cannon, Querido, Britton and Bright (1927) have stated that these two components of chemical temperature regulation constitute first and second lines of defense in the protection of the warm blooded animal against cold, with the hormonal mechanism forming the first line of defense and shivering the second. They have noted marked symptoms of sympathetic activity when cats with denervated hearts were cooled by intragastric ice water and observed a rise in metabolic rate of normal men without shivering when cooled in the same way. Since, as shown by Boothby and Sandiford (1923) and others, epinephrine injections increase metabolic rate it is logical to assume a hormone control of temperature regulation which functions to protect against cold. Swift (1932) observed an average increase of metabolic rate of men of 11.3 per cent when exposed to cold without shivering but these individuals experienced "increased muscle tension" which Swift considered to be part of the shivering mechanism. Swift could find no change in blood sugar during the exposure to cold and cited this as evidence opposing Cannon's theory. More recently Burton and Bronk (1937) have made similar investigations on anesthetized cats and have taken, as their criterion of shivering, action currents from the muscles. They found no increase in metabolism without evidence of muscular activity.

Recently we have concluded an extended series of observations on the effect of anesthesia by members of the barbital group on shivering. Bar-

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bital anesthetics are particularly depressant on temperature regulatory functions especially shivering. Having these trained dogs at our disposal we have investigated the change in oxygen consumption rate when normal trained dogs are slowly cooled and when shivering occurs. Dogs possess an advantage over human individuals in that they are not disturbed by subjective influences which in a man can effect shivering. For example, a human subject can voluntarily repress incipient shivering or can voluntarily imitate shivering movements. A trained dog not knowing the type of experimentation being observed is less likely to act voluntarily to aid or inhibit the start of shivering. The dogs were carefully trained and chosen and were used in a basal state without anesthesia.

It is quite obvious that when the two components of chemical temperature regulation are operating simultaneously in an extremely cold environment the two cannot be separated or evaluated. It is necessary to choose conditions wherein only one component is active. According to Cannon, Querido, Britton and Bright (1927) the hormonal component is first brought into play and is followed by shivering. Hence slow cooling of an animal should first initiate hormonal regulation to be followed later, when the cooling is more intense, by shivering. Rapid cooling might bring both factors into action simultaneously and separate effects could not be determined.

One of the most controversial phases of the problem is the measurement of shivering. This has been measured in three different ways as follows: 1, visual inspection; 2, mechanical shivering recorders (Swift, 1932, and Hemingway, 1940) and 3, measurement of muscle action currents (Burton and Bronk, 1937). Of these methods visual inspection is likely to be the least reliable and mechanical recorders which depend on limb movements may not reveal contractions of small muscle units or muscle tension changes. For these reasons the electrical method is the one of choice provided sufficiently high amplification can be obtained.

With these considerations in mind we have conducted experiments on dogs which were slowly cooled in an electrically shielded cooling cabinet. The onset of shivering was measured electrically, mechanically and visually and metabolic rate, temperature, respiratory and cardiac rate measurements were made under basal, cooling and shivering conditions.

Experimental. From twenty short haired dogs three were chosen for their ability to lie quietly in the metabolism apparatus for 2 to 4 hour periods. The animals were carefully trained for metabolism measurements and were used for the shivering experiments only when control experiments of two hours' duration showed uniform oxygen consumption rates. On the day of the experiment the fasting animal was brought to the metabolism room and required to lie quietly at rest for 2 to 3 hours. The dog was then placed in the apparatus, as shown in figure 1, with his head

sealed in a head mask through which air circulated from a metabolism apparatus. The body of the dog and head mask were placed in a double

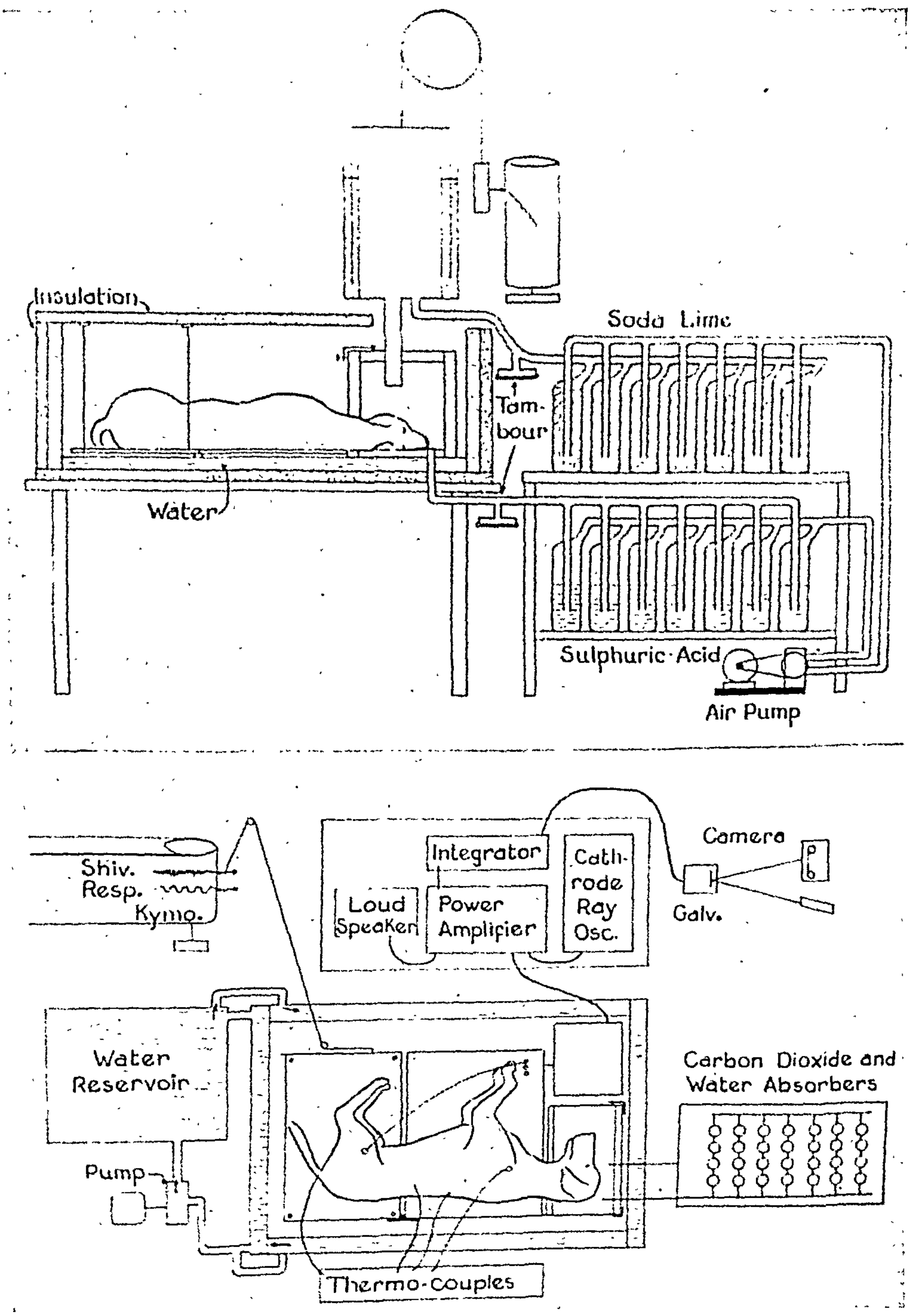

Fig. 1. Metabolism apparatus for measuring metabolic rate before and after onset of shivering.

walled sheet iron chamber which acted as an electrical shield. The temperature of the chamber was controlled by regulating the temperature of

water flowing from a reservoir through the hollow metal walls. In this way reproducible and finely adjusted cooling rates could be obtained. Rectal and skin temperatures were measured by thermocouples. The forepart of the dog including the thoracic region rested on a fixed platform while the hind legs rested on a movable platform suspended from wires and hinged to the fixed platform as shown in figure 1. Shivering was recorded by movements of the suspended platform. Small electrodes were placed on the skin above muscles which were chosen for their vigorous shivering movements when the animal shivered. These electrodes were connected to a preamplifier placed within the animal chamber and the preamplifier was connected with a power amplifier placed outside. Connected with the power amplifier there were a loud speaker, a cathode ray oscilloscope and an integrator. The integrator which served to rectify and summate the rapidly varying action potentials was connected to a recording galvanometer and produced records similar to that of figure 2. Oxygen consumption, carbon dioxide and respiratory water production

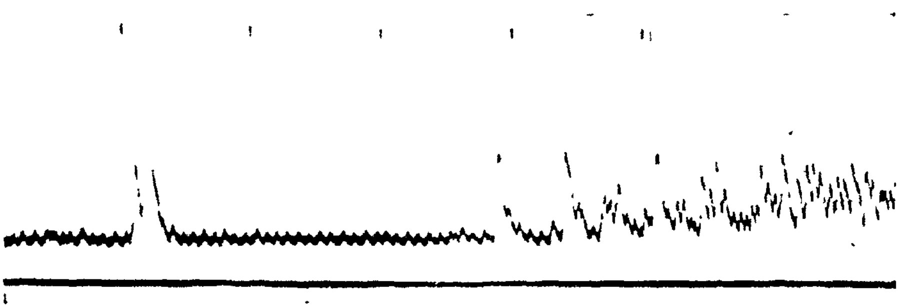

Fig. 2. Record of integrated action potentials showing abrupt onset of shivering. Time intervals one minute.

were measured by a closed circuit respiratory metabolism apparatus using soda lime and sulphuric acid absorbers. A refrigerator pump circulated air through the closed system and interchangeable sets of absorbers permitted metabolism measurements to be made in several periods.

The first experiments consisted of controls with the metabolism being measured in 20 to 30 minute periods during the course of 2 to 3 hours with the chamber temperature set at 28 to 30 degrees. In the course of these successive periods on any one day the variation of oxygen consumption rate was within ±2 per cent although the day to day variation was greater. When shivering was to be produced the metabolism, in a control period at 28 to 30 degrees, was first measured. The absorption bottles were then changed and cool water was passed through the hollow walls of the chamber at such a rate that the chamber temperature dropped 5 to 6 degrees in 20 to 40 minutes. During this period designated as the cooling period all of the indicators were carefully watched for shivering which included the electrical measuring devices, the mechanical recorder and simply visual observation. At the first sign of shivering the absorption bottles were

again changed for the third or shivering period and the experiment was terminated after 15 to 30 minutes of shivering. Each entire experiment occupied four to five hours.

RESULTS. *The onset of shivering.* The onset of shivering was readily detected either by the cathode ray oscilloscope, the loud speaker or the integrator. Visible shivering and movements of the mechanical recorder usually followed within one minute. The onset of shivering was usually abrupt. In some cases short periods of shivering would be separated by quiscent intervals but as cooling continued the quiescent periods became progressively shorter in duration. There was no indication of a gradual

TABLE 1

Basal metabolic rates of dogs used in experiments compared with normal basal metabolic rates previously reported

Basal metabolism values

DOG	WEIGHT	SEX	OXYGEN CONSUMPTION RATE	CALORIES PER SQ. M. PER HOUR
	kilos		*liters per hour*	
A	9.3	F	2.89	28.2
B	13.0	M	4.40	34.3
C	7.5	F	3.56	39.7
Average				34.0

Normal basal metabolism values as given by other investigators

OBSERVERS	WEIGHT OF DOGS	CALS./SQ.M./HR.
Kitchen (1923)	10–15	40.3
Kunde (1926)	10–13	32.4
De Beer and Hjort (1938)	20–27	43.6
Lusk and DuBois (1924)		32.2
Morgulis (1924)	7–8	43.6
Average		38.4

rise in the integrated and rectified action potentials away from the base line before shivering commenced. The sudden onset of shivering is shown in figure 2.

Basal metabolism. The weight and sex of the three dogs used with their basal metabolism values are given in table 1. The heat production in calories per square meter per hour has been computed using Meeh's formula and assuming a value of 4.82 for the calorific value of a liter of oxygen. The basal metabolism values are in the range considered normal by the investigators listed in table 1. The dog A had an exceptionally low basal metabolic rate but this was to be expected because she was an old female, somewhat obese and very coöperative in her ability to rest in a relaxed state. The average basal metabolism of the three dogs, 34.0 calories per

square meter per hour, was below the average values which have been given by various investigators as normal values.

Rise in metabolic rate as a result of cooling without shivering. In table 2 are listed the relative values of the oxygen consumption rate of the pre-shivering cooling period followed by the shivering period. The control period (basal) value is arbitrarily assigned the comparative value of 100. There was always a slight rise in oxygen consumption rate which varied from 0 to +22 per cent with averages for each dog as shown in table 2. The averaged values varied from +5 to +10 per cent. When shivering started the increases of metabolic rate were much more pronounced and of course would have increased with further cooling.

TABLE 2

Increase of metabolic rate on cooling

DOG	SEX	NUMBER OF EXPERIMENTS	BASAL		COOL		SHIVERING	
			O_2	R.Q.	O_2	R.Q.	O_2	R.Q.
A	F	6	100	0.88	106	0.87	142	0.80
B	M	7	100	0.82	110	0.87	128	0.94
C	F	6	100	0.83	105	0.86	124	0.86
Average........		19	100	0.84	107	0.87	130	0.89
Control.........		10	100	0.82	98	0.82	101	0.84

Discussion. *Rôle of two components of chemical temperature regulation.* It is evident from the results obtained in table 2 that the non-shivering component of chemical temperature regulation is so small that it is practically without significance when an animal is exposed to cold under the conditions described. An increase of basal metabolic rate of less than 10 per cent can contribute very little in defense against cold and is unimportant when compared with the increases of metabolism due to voluntary movements and shivering. It is possible that the non-shivering component of chemical temperature is even less than that measured since the skin electrodes may not have picked up action currents from individual muscle fibers which contracted before larger muscle fiber groups became active. On the other hand the abrupt onset of shivering as shown in figure 2 is evidence against this since if shivering commences by a progressively increasing number of fibers becoming active then one could expect a slowly rising integrated action potential instead of the abrupt rise seen in figure 2. Hence it may be concluded that within the limitations of experimentation the results indicate that a non-shivering component of chemical temperature regulation exists but is without practical significance in protecting against exposure to cold.

The increase of basal metabolic rate which occurs after a prolonged exposure to cold has been observed by many investigators, e.g., Horvath,

Hitchcock and Hartman (1938), Horst, Mendel and Benedict (1933) and Gelineo (1934). This effect which may explain the seasonal variation of basal metabolic rate is evidently acquired as a result of acclimatization to a cold environment and requires a considerable period of time for its development. If an animal is exposed to cold without acclimatization, the non-shivering component of chemical temperature regulation is without appreciable value and the protection against cold depends practically on shivering. These results are suggestive of a general plan for temperature control of warm blooded animals in which a nervous mechanism (shivering) protects against a sudden exposure to cold whereas the hormone mechanism (thyroid-adrenal) functions as a result of prolonged exposure to cold and may explain acclimatization, see Horvath, Hitchcock and Hartman (1938).

SUMMARY

The oxygen consumption rate and CO_2 production of three trained dogs have been measured while the animals were slowly cooled in an electrically shielded metabolism chamber. The onset of shivering was noted by electrical, mechanical and visual methods. In the electrical method the action currents were picked up by small skin electrodes placed over shivering muscles. The action potentials were amplified and made to operate a loud speaker, a cathode ray oscilloscope and a recording electronic integrator. On cooling before shivering started there was an average increase of metabolic rate of 7 per cent over basal. During the first 20 minutes of shivering the increase over basal was 30 per cent. These results indicate that the increase of metabolic rate without shivering has little effect in combating exposure to cold.

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THE EFFECT OF MAGNESIUM DEFICIENCY ON THE EXCITABILITY OF THE VAGO-INSULIN AND SYMPATHETICO-ADRENAL SYSTEMS[1]

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In a series of papers Gellhorn and his collaborators, Cortell, Feldman and Kessler, have developed methods which allow one to investigate the excitability of the autonomic centers regulating the secretion of adrenalin and insulin in the rat. It was shown that drugs such as metrazol produce a hyperglycemia in the normal rat and a hypoglycemia in the adreno-demedullated rat. If, however, the drug is injected in the adreno-demedullated-vagotomized rat the blood sugar remains unchanged. These experiments were interpreted to mean that the drug acts on both sympathetico-adrenal and vago-insulin systems and that, in the normal animal, the former predominates over the latter. That metrazol, as well as other procedures, such as the administration of a convulsive shock to rats, give a quantitative estimate of the excitability of the autonomic systems was shown in studies on the effect of the thyroid hormone. It could be shown that the thyroidectomy lowered the excitability of the centers of the sympathetico-adrenal system without affecting the vago-insulin system. The injection of thyroxin increased the excitability of the sympathetico-adrenal system, but did not alter the vago-insulin system.

The present paper is an attempt to study the effect of variations in the ionic balance of the body on the excitability of the autonomic centers. From the studies of Kruse, Orent and McCollum, as well as of Greenberg and collaborators, it is known that Mg deficiency causes disturbances in the function of the central nervous system indicated by vasodilatation, increased irritability, and tonic-clonic convulsions. Greenberg observed that the susceptibility of Mg deficient rats to picrotoxin convulsions is greater than that of normal rats. Since earlier observations showed that picrotoxin acts similarly to metrazol these studies suggest that Mg deficiency alters the excitability of autonomic centers. The experiments reported in this paper show, indeed, that that is the case.

[1] Aided by a grant from The John and Mary Markle Foundation; assistance given by the WPA Project no. 30278.

METHOD. A diet similar to that used by Greenberg and collaborators was chosen. We used, however, adult rats and subjected them to a much milder degree of Mg deficiency than was used by the previously named authors. Correspondingly, the symptoms were very slight. The only constant effect of the diet given over several weeks was a roughing of the fur, and a loss in weight. The experiments were carried out on vagotomized and on adreno-demedullated rats. *Fifty-five milligrams of metrazol* per kilo was injected subcutaneously. An electric shock was applied to the head for 0.5 second using a General Electric Variac supplied with the 60 c.p.s. current as source of stimulation (cf. Kessler and Gellhorn). The blood sugar was determined with the Somogyi modification of the Shaffer-Hartman method.

RESULTS. The experiments were performed on two groups of rats, the first being kept on a diet containing 5.3 magnesium/100 grams food; the second on a similar diet containing only 3.7 mg magnesium/100 grams food.

Table 1 shows that vagotomized rats kept on these diets for one month show *a greatly increased hyperglycemia on injection of metrazol.* The differences between the vagotomized animals kept on control diet and the Mg deficient rats are statistically significant. The effect of the two diets is practically identical. Since, in the vagotomized rats, the rise in sugar is due exclusively to the liberation of adrenalin through the sympathetic system, the experiments seem to indicate that the centers of the sympathetico-adrenal system are in a state of a greatly increased excitability when the Mg content of the diet is reduced.

In contradistinction to these results, table 1 shows that adreno-demedullated rats do not show any significant changes in the blood sugar when injected with metrazol, although the rats on a control diet show regularly a fall in blood sugar under these conditions. Since the hypoglycemia in adreno-demedullated rats kept on a control diet and subjected to metrazol is due to the liberation of insulin through discharges mediated by the vagus (Feldman, Cortell and Gellhorn), the experiments suggest that the Mg deficiency of the diet caused a marked reduction in the excitability of the vago-insulin system.

Since it has been found previously that thyroidectomy distinctly lowers the excitability of the centers of the sympathetico-adrenal system, it was thought to be of interest to investigate whether this effect could be overcome by Mg deficiency. Consequently, thryoidectomized, vagotomized rats were kept on a Mg deficient diet and then tested with metrazol. Table 1 shows that the Mg deficiency displays its characteristic effect (increased hyperglycemic reaction to metrazol) in spite of the absence of the thyroid gland. The hyperglycemic reaction is, however, not quite as large as was observed in the Mg deficient animals in which the thyroid was intact.

An effort was made to determine whether similar results would be ob-

tained if the excitability of the autonomic centers were tested with the electroshock method. This procedure was applied on vagotomized and on

TABLE 1

Effect of metrazol (55 mgm/kgm.) on blood sugar of Mg-deficient rats

I. Vagotomized rats

BLOOD SUGAR, MGM. PER CENT	CONTROLS,* MEAN	I*					II†				
		1	2	3	4	Mean	1	2	3	4	Mean
minutes											
0	74.2±2.0	78.5	80.6	77.4	75.2	77.9±1.9	77.7	79.5	81.1	78.5	79.2±1.3
15	95.9±3.4	99.9	95.6	98.9	93.5	97.0±2.6	101.0	96.8	94.6	95.6	97.0±2.5
30	112.4±3.8	126.8	122.5	131.1	124.7	126.3±3.2	129.0	135.5	139.7	127.9	133.0±4.7
45	123.1±5.8	150.5	142.9	146.2	146.2	146.5±2.7	151.3	150.5	150.5	141.9	148.6±3.9
60	103.6±4.1	140.8	133.9	135.5	130.1	135.1±3.8	138.6	145.1	142.9	135.5	140.5±3.7

II. Adrenodemedullated rats

BLOOD SUGAR, MGM. PER CENT	CONTROLS,* MEAN	I‡					II§				
		1	2	3	4	Mean	1	2	3	4	Mean
minutes											
0	61.8±3.2	68.8	64.5	67.7	68.8	67.5±1.8	66.6	68.8	64.4	69.8	67.4±2.1
15	50.6±3.9	69.8	68.8	70.9	68.8	69.6±0.9	68.8	66.6	75.2	79.5	72.3±5.1
30	49.4±4.5	66.6	75.2	79.5	83.8	76.3±6.4	84.9	76.3	68.8	78.5	77.1±5.8
45		60.2	72.0	64.5	78.5	68.8±7.0	74.1	76.3	60.2	70.9	70.4±6.2
60	56.0±2.5	64.5	67.7	67.7	70.9	67.7±2.3	69.8	67.7	64.4	68.8	67.7±2.0

III. Thyroidectomized-vagotomized rats§

BLOOD SUGAR, MGM. PER CENT	CONTROLS,* MEAN	II§						
		1	2	3	4	5	6	Mean
minutes								
0	74.1±1.2	77.4	79.5	77.4	76.3	78.5	76.3	77.6±1.1
15	92.6±4.7	91.3	98.9	95.6	92.5	92.5	96.8	94.6±2.7
30	102.2±4.1	126.8	118.2	116.1	122.5	124.7	131.1	123.2±5.1
45	104.7±4.8		137.6	136.5	134.4	138.6	146.2	138.4±4.0
60	96.0±4.6		126.8	129.0	124.7		136.5	129.2±4.4

* Controls: Rats on normal diet.
On diet I (Mg content 5.3 mgm/100 grams) for 29 days.
† On diet II (Mg content 3.7 mgm./100 grams) for 29 days.
‡ On diet I for 26 days.
§ On diet II for 28 days.

adreno-demedullated rats subjected to the Mg deficient diet and the results thus obtained were compared with those observed on animals kept under control conditions. Table 2 shows the results, which are very similar to

those obtained in the experiments with metrazol. The hyperglycemic reaction following the electric shock in vagotomized and Mg deficient animals is significantly increased over and above that of the control animals. On the other hand, it is found that adreno-demedullated Mg deficient rats failed to show a fall in blood sugar after administration of the electric shock, although animals on a control diet show this effect regularly.

After these experiments had been completed the animals were returned to a normal diet and kept on this diet up to 62 days. During that time they were tested several times with metrazol, as shown in table 3. The return to the control diet had only a very slight, if any, effect on the appearance of the rats. The experiments with metrazol showed that even after 62 days the results were practically identical with those obtained at the end of the Mg deficiency period. Similarly, it was found, as indicated by the tests performed on the adreno-demedullated rats, that metrazol failed to elicit any hypoglycemia in these animals one or two months after they had been returned to the control diet.

TABLE 2

Effect of electrical shock (0.5 sec.) on the blood sugar of Mg-deficient rats

I. Vagotomized rats

BLOOD SUGAR, MGM. PER CENT	CONTROLS, MEAN	I*					II†				
		1	2	3	4	Mean	1	2	3	4	Mean
minutes											
0	77.4±1.4	75.2	78.5	80.6	78.5	78.2±1.9	79.5	77.4	80.6	76.3	78.5±1.7
10	97.1±1.7	99.9	101.0	96.8	97.8	98.9±1.7	98.9	101.0	99.9	96.8	99.2±1.5
40	119.3±2.0	127.9	130.1	124.7	123.6	126.6±2.6	126.8	132.2	127.9	125.7	128.2±5.1
70	106.2±4.0	122.5	120.4	118.2	117.1	119.6±2.1	121.5	124.7	116.1	117.1	119.9±3.5

II. Adreno-demedullated rats

BLOOD SUGAR, MGM. PER CENT	CONTROLS, MEAN	I*				II†				
		1	2	3	Mean	1	2	3	4	Mean
minutes										
0	63.4±2.1	64.5	68.8	66.6	66.6±1.8	68.8	66.6	66.6	67.7	67.4±0.9
10	55.5±3.5	62.3	67.7	63.4	64.5±2.3	64.5	66.6	62.3	66.6	65.0±1.8
40	43.5±5.6	62.3	70.9	61.2	63.8±4.4	60.2	67.7	68.8	69.8	66.6±3.8
70	64.4±4.0	62.3	68.8	64.5	65.2±2.7	65.5	68.8	72.0	66.6	68.2±2.5

* On diet I (Mg content 5.3 mgm./100 grams) for 33 days.
† On diet II (Mg content 3.7 mgm./100 grams) for 36 days.

It was tentatively assumed that the effect of Mg deficiency on the sympathetico-adrenal and vago-insulin systems of the rat was due to an alteration in the excitability of the centers of these systems. It was, however, not ruled out by the experiments described thus far that the amount of adrenalin and insulin secreted in the vagotomized and adrenodemedullated

TABLE 3

Effect of metrazol on blood sugar (mgm. per cent) of magnesium deficient rats returned to control diet

A. Adrenodemedullated rats

TIME	ON CONTROL DIET FOR										
	14 days*				30 days*	62 days*	21 days†				
	1	2	3	Mean, standard deviation	1	1	1	2	3	4	Mean, standard deviation
minutes											
0	70.9	68.8	67.7	*69.1* ± 1.3	68.8	66.6	68.8	65.5	66.6	68.8	*67.4* ± 1.4
15	68.8	67.7	64.5	*67.0* ± 1.8	83.8	68.8	74.1	64.4	68.8	68.8	*69.0* ± 3.4
30	67.7	67.7	65.5	*66.9* ± 1.0	79.5	74.1	72.0	60.2	72.0	75.2	*69.8* ± 5.7
45	64.5	66.6		*65.5* ± 1.1	70.9	70.9	72.0	66.6	70.9	70.9	*70.1* ± 2.1
60	67.7	68.8		*68.2* ± 1.7	66.6	70.9	69.8	65.5	66.6	69.8	*67.9* ± 1.9

B. Vagotomized rats

TIME	ON CONTROL DIET FOR													
	14 days*					30 days*				62 days*				21 days†
	1	2	3	4	Mean, standard deviation	1	2	3	Mean, standard deviation	1	2	3	Mean, standard deviation	1
minutes														
0	77.4	80.6	79.5	78.5	*79.0* ± 1.2	77.7	78.5	76.3	*77.5* ± 0.9	74.1	77.4	78.5	*76.7* ± 1.9	77.4
15	99.9	103.2	96.8	98.9	*99.7* ± 2.3	94.5	93.5	96.8	*94.9* ± 1.4	99.9	101.0	96.8	*99.2* ± 1.8	101.0
30	118.2	124.7	130.1	129.0	*125.5* ± 4.7	120.4	127.9	126.8	*125.0* ± 3.3	129.0	137.6	133.9	*133.5* ± 3.5	133.9
45	151.3	149.4	155.8	149.4	*151.5* ± 2.6	140.8	140.8	138.6	*140.0* ± 1.0	139.7	144.0	140.9	*141.5* ± 1.8	144.0
60	129.0	129.0	127.9	124.7	*127.7* ± 1.8	129.0	130.1	129.0	*129.4* ± 0.5	127.9	132.2	130.1	*130.1* ± 1.7	129.0

* Rats of group I.
† Rats of group II.

TABLE 4

Effect of adrenalin and insulin on the blood sugar of Mg-deficient rats

BLOOD SUGAR, MGM. PER CENT	CONTROLS, MEAN	I*				
		1	2	3	4	Mean
I. Adrenalin (1 cc. 1:25,000/kgm. intraperitoneally). Vagotomized rats						
minutes						
0	77.6 ± 1.3	79.5	81.1	77.4	78.5	79.1 ± 1.4
30	109.9 ± 3.0	110.9	107.5	118.2	105.3	111.2 ± 4.9
60	83.5 ± 2.5	87.0	86.0	84.9	89.2	86.8 ± 3.2
II. Insulin (0.01 unit/100 gram intraperitoneally). Adrenodemedullated rats						
0	66.8 ± 1.4	68.8	65.5	67.7	66.6	67.2 ± 1.2
60	43.7 ± 1.4	43.0	40.8	44.0	45.1	43.2 ± 1.6

* On diet I for 36 days.

rats respectively, might have been unaltered and that the significant changes in the response of the blood sugar to metrazol and electrical shock might be attributed to an alteration in the sensitivity of peripheral structures (liver, muscles) to insulin and adrenalin. Therefore, adrenalin was injected into vagotomized and insulin into adreno-demedullated rats, and the effect of the injection of these hormones on the blood sugar was tested on animals on control and on a Mg deficient diet. The experiments recorded in table 4 show conclusively that the Mg deficiency in the diet has no effect whatever on the hyperglycemic effect of adrenalin and on the hypoglycemic effect of insulin. It must, therefore, be concluded that the increased hyperglycemia in vagotomized rats is due to a sensitization of the center of the sympathetico-adrenal system. The absence of the hypoglycemic effect in adreno-demedullated rats indicates a decrease in the sensitivity of the center of the vago-insulin system. It is interesting to note that the decrease in the sensitivity of the centers of the vago-insulin system is apparently not restricted to this branch of the parasympathetic system. It was found that whereas normal rats subjected to a convulsive shock regularly urinated during the convulsions, the Mg deficient animals failed to do so. They show, however, during the period of catalepsy following the shock a decrease in pulse rate similar to that found in control animals.

SUMMARY

The effect of a magnesium deficiency in the diet on the centers regulating the discharge of adrenalin via the sympathetic system and the secretion of insulin through the vagus was studied in vagotomized and in adreno-demedullated rats. It was found that metrazol and electrically produced convulsive shocks produced a greater hyperglycemia in vagotomized magnesium deficient rats than in control animals. On the other hand, adreno-demedullated rats react to these tests with a distinct hypoglycemia, whereas magnesium deficient adreno-demedullated rats show no change in the blood sugar. Since magnesium deficiency does not alter the effect of adrenalin and insulin on the blood sugar, the experiments indicate a marked shift in the balance of the autonomic centers under the influence of magnesium deficiency. The centers of the sympathetico-adrenal system become greatly sensitized whereas the centers of the vago-insulin system fail to respond to stimuli which are effective in control animals. The effects persist for weeks after the animals have been returned to the control diet.

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THE MECHANISM OF THE COAGULANT ACTION OF DABOIA VENOM

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The coagulant power of the venom of the Daboia (Russell's viper) has been known for many years (1), but there have been numerous conflicting reports concerning the mechanism of its action. Lamb (1) found that whole blood or recalcified plasma was clotted by the venom in dilutions up to 1/1,000,000, whereas citrated plasma was clotted only by dilutions approximating 1/2,000 or stronger. The action of the venom *in vivo* was found to resemble that described by Wooldridge (2) for tissue extracts. Many subsequent investigators (3, 4, 5, 6, 7, 8) have verified the coagulant power of the venom for whole blood or recalcified plasma. The effect of the venom on citrated preparations has been disputed, some (4, 5, 6, 9) finding it to be coagulant, in high concentrations at least, while others (3, 8, 10, 11) obtained negative results. Arthus (3) observed that the venom did not behave either as a prothrombin or a thrombin, but resembled thromboplastin in its action. Houssay and Sordelli (4) demonstrated that the full coagulant effect of the venom required the presence not only of calcium ions but of thromboplastin and of serum (apparently as a source of prothrombin) as well. They concluded that the venom accelerated coagulation by "facilitating the formation of thrombin". Ganguly (8) found that the venom accelerated prothrombin conversion in the presence of platelets but not in their absence; he concluded that the venom hastened platelet lysis.

Eagle (11) studied the coagulant action of numerous snake venoms and offered the conclusion that coagulant venoms "are of two types; one which, like trypsin and like the calcium-platelet system, acts on prothrombin to form thrombin; and one which, like papain and thrombin, acts directly on fibrinogen to form fibrin." Eagle, employing only citrated plasma as a coagulating medium, did not detect any coagulant power in Daboia venom

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and did not pursue the possibility that it might represent a type of coagulant venom unlike those previously described. The work of Houssay and Sordelli foreshadowed this possibility, and the experiments presented herewith lend it further support.

MATERIALS AND METHODS. *Daboia venom.* Daboia venom was supplied[2] in 0.5 mgm. ampoules of dried material. Each ampoule was accompanied by a 5 cc. vial of 0.5 per cent phenol in distilled water, in which the usual dilution of 1/10,000 was made when the venom was to be used. Further dilutions of the venom were made in 0.9 per cent NaCl.

Plasma. Plasma for routine experiments was obtained from cows, by collecting blood in a paraffined pail containing enough 10 per cent sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$) to make a final blood concentration of 0.6 to 0.9 per cent citrate. Less citrate not infrequently failed to prevent spontaneous coagulation. The blood was immediately chilled and centrifuged in a cold room for 1½ hours at 1200 to 1500 r.p.m. *Platelet-free plasma* was obtained by recentrifuging portions of the crude plasma twice more in the cold room for one hour at 2500 r.p.m., after which this clarified plasma was immediately passed through a Berkefeld "V" filter to remove the few remaining platelets.

For certain experiments, plasma was obtained from horses or from humans by venipuncture or from rabbits by cardiac puncture. These bloods were mixed at once with about 1/10 volume of 3.5 per cent sodium citrate, making a final citrate concentration of 0.35 to 0.4 per cent; in other respects the preparation followed that of bovine plasma.

Thromboplastic substances. Thromboplastic substances ("thromboplastins") of the following types were employed:

Human placental coagulant (12) was made up in fresh lots each week in this laboratory, lots T. G. 433 to 460 being used in the experiments described.

Bovine brain extract (prepared according to the method of Eley *et al.* (12)) was made up in two lots (T. G. 294 and 297) about two years previous to the experiments described and kept in the cold room until use.

Fresh purified cephalin, kept under alcohol until use, was prepared according to the method of Renall (13) and furnished through the kindness of Dr. H. C. Christensen of the department of biochemistry, Harvard Medical School. A two-year-old sample of cephalin (prepared according to Maltaner's modification (14) of the method of Levene (15)) was also employed, and a fresh, highly purified sample of lecithin (furnished by Dr. Christensen) was used as a control for the cephalin preparations.

Bovine platelets were obtained from the sediment of the recentrifuged plasma. They were washed by suspension in chilled citrated sodium chloride (1 part 3.5 per cent sodium citrate to 8 parts 0.9 per cent NaCl) and a centrifugation at 2500 r.p.m. They were then re-suspended in ci-

[2] Through the courtesy of Burroughs, Wellcome & Co., New York.

trated saline and centrifuged for 5 to 10 minutes at about 600 r.p.m. to sediment the red and white blood cells. This step was repeated several times until the supernatant contained not more than one red or white blood cell per 2,000 platelets. The platelet preparation was completed in not over six hours from the time of collecting the blood. The platelets so prepared showed but little disintegration in the next 48 hours and were very active in hastening coagulation.

Commercial coagulants. "Thromboplastin—Squibb", "Coagulen—Ciba", "Neo-Hemoplastin—Parke, Davis", and "Fibrogen—Merrell", were tested for their activity in comparison to the coagulants described above.

Fibrinogen. Fibrinogen was prepared by repeated precipitation (at 0–2°C. and pH 6.5) with $\frac{1}{3}$ volume of saturated ammonium sulfate. A 0.35 per cent concentration of sodium citrate was maintained in all reagents at all stages of the preparation. After three or four precipitations the fibrinogen was virtually free of prothrombin; it was then dissolved in 5 per cent NaCl containing 0.35 per cent sodium citrate and stored in a refrigerator until use. Suitable portions were diluted before use with 5 volumes of distilled water.

Prothrombin. Prothrombin was prepared by Green's modification (16) of Mellanby's method (17) and was kept in 0.9 per cent NaCl and 0.35 per cent sodium citrate in the refrigerator. A typical preparation of prothrombin, containing 0.59 mgm. N/cc., when converted to thrombin by 1 to 4 minutes' incubation with 0.1 per cent $CaCl_2$ and a trace of thromboplastin, would clot 10 volumes of citrated fibrinogen in 8 to 12 seconds at 37°C.

Calcium chloride. Calcium chloride was employed in a 2.5 per cent or (in some experiments) in a 1.1 per cent solution. The amount added was in all instances that which had been found to produce the most rapid coagulation of the preparation employed.

All experiments were carried out in 8 mm. glass flocculation tubes in a water bath at 37°C. Unless otherwise specified, each test tube contained one-half cubic centimeter of plasma, optimal $CaCl_2$ solution as stated above, such other reagents as are indicated in the protocols, and sufficient 0.9 per cent NaCl to bring the final volume up to one cubic centimeter. Clotting times were measured from the time of adding the last reagent ($CaCl_2$ or thrombin solutions) to the time when the contents of the tube ceased to flow on tilting.

RESULTS. *Action of Daboia venom on citrated and on recalcified preparations.* Daboia venom was added to citrated fibrinogen and to citrated plasma in concentrations of 0.03 mgm. per cc., and of 0.14 mgm. per cc. No evidence of coagulation was noted throughout the periods of observation. Control preparations were clotted rapidly by thrombin (see table 1).

Daboia venom, 0.01 mgm. per cc., was added to samples of recalcified

plasma from various animal species. Controls were clotted by recalcification alone. On addition of Daboia venom the various types of plasma clotted from 4 to 12 times faster than on recalcification alone (see table 2).

Action of Daboia venom on recalcified platelet-free plasma. Platelet-free plasma, prepared by Berkefeld filtration as described above, showed a recalcification time of from 1 to 12 hours, varying with different preparations. If Daboia venom (0.01 mgm.) was added before recalcification, the clotting time was reduced to 60 to 90 seconds. This acceleration was repeatedly observed, using different lots of plasma and varying amounts of Daboia venom. It was invariably found, however, that though the addition of venom greatly accelerated the coagulation rate of filtered plasma, the clotting time obtained was never as short as that produced by the venom in unfiltered (control) plasma. In this respect the action of Daboia venom differed from that of tissue extract, which clotted platelet-free plasma in essentially the same time as it clotted the unfiltered control. Typical results are summarized in table 3.

TABLE 1

Action of Daboia venom on citrated fibrinogen and plasma

SUBSTRATE	COAGULANT			
	Daboia venom		Thrombin solution	
	Amount	Clotting time	Amount	Clotting time
	mgm.		*cc.*	*seconds*
Fibrinogen	0.03	No clot in 20 minutes	0.3	35
Horse plasma	0.03	No clot in 20 minutes	0.3	30
Horse plasma	0.14	No clot is 2 hours	0.15	60

TABLE 2

Action of Daboia venom on recalcified plasma

SOURCE OF PLASMA	CLOTTING TIME ON ADDITION OF:	
	$CaCl_2$	$CaCl_2$ + 0.01 mgm. Daboia venom
	minutes	*seconds*
Horse	6	30
Cow	7	70
Rabbit	2	29
Man	3	26

Inability of Daboia venom to substitute for prothrombin. The observations of Arthus (3), of Houssay and Sordelli (4) and of Ganguly (8), and those presented above could be explained by the assumption that Daboia venom functions as a prothrombin, if it were further assumed that the reagents employed in these experiments were so nearly free of thromboplastic substances that the conditions for thrombin formation had not prevailed. This possibility was therefore tested as follows:

1. *Incubation of Daboia venom with tissue extract and calcium chloride.* One one-hundredth milligram Daboia venom in 1 cc., plus 0.25 mgm. T. G.

294 and 0.1 cc. of 1.1 per cent $CaCl_2$ solution, incubated at 37°C., developed no power to clot fibrinogen during 1½ hours of observation.

Control: one cubic centimeter prothrombin solution, plus T. G. 294 and $CaCl_2$ solution as above, after 1 minute and 41 seconds' incubation developed the power to clot 5 volumes of fibrinogen in 9 seconds.

2. *Influence of adding Daboia venom to prothrombin: Rate of thrombin formation and potency of thrombin formed.* The possibility existed that Daboia venom could function as a prothrombin only in the presence of natural prothrombin. To test this possibility, varying amounts of tissue extract and of the venom were incubated with prothrombin and $CaCl_2$ and the potency of the thrombin formed and the time required to develop a given potency were noted. The results, presented in table 4, indicated that the venom—like tissue extract—accelerated the formation of thrombin but did not significantly affect the maximum amount of thrombin obtainable.

TABLE 3

Action of Daboia venom on recalcified Berkefeld-filtered (platelet-free) plasma and on unfiltered plasma; comparison with tissue extract

PLASMA	CLOTTING TIME ON ADDITION OF: $CaCl_2$	$CaCl_2$ + 0.01 mgm. Daboia Venom	$CaCl_2$ + optimal tissue extract (prothrombin time)
	minutes	*seconds*	*seconds*
Bovine: unfiltered	7	70	33
Bovine: filtered	Overnight	170	34
Equine: unfiltered	6	30	33
Equine: filtered	60	75	32

TABLE 4

Influence of Daboia venom on conversion rate of prothrombin and on potency of thrombin formed; comparison with tissue extract

COAGULANT 1 CC. PROTHROMBIN + 0.2 CC. OF 1.1% $CaCl_2$ +	MAXIMUM POTENCY THROMBIN MIXTURE CLOTTED FIBRINOGEN IN*	CONVERSION TIME MAXIMAL POTENCY FIRST REACHED IN
	minutes	*minutes*
(No coagulant added)	6†	20†
	seconds	
Daboia venom, 0.001 mgm.	11	30
Daboia venom, 0.01 mgm.	9	20
Daboia venom, 0.03 mgm.	9	9
T. G. 294, 0.015 mgm.	11	4
T. G. 294, 0.25 mgm.	9	1⅔
T. G. 294, 0.015 mgm. + Daboia venom, 0.01 mgm.	10	2

* One-tenth cubic centimeter of thrombin mixture added to 0.5 cc. of fibrinogen.

† Maximal potency not obtained during period of observation.

3. *Daboia venom as a substitute for prothrombin in plasma with a subnormal prothrombin content.* To test the possibility that Daboia venom might function as a prothrombin substitute only in the presence of the normal constituents of plasma, the action of the venom was observed on plasma preparations containing varying percentages of the normal prothrombin. These were prepared by mixing appropriate proportions of Berkefeld-filtered plasma with plasma rendered virtually prothrombin-free by alumina absorption (18). The "prothrombin time" (19) for each mixture was determined by the addition of the optimal amount of tissue extract and $CaCl_2$ solution. Daboia venom (0.01 mgm.) was then added to samples of each mixture; tissue extract and $CaCl_2$ were added as before, and the clotting time noted (see table 5). It was found that 0.01 mgm. Daboia venom added to the 20 per cent-prothrombin mixture gave a clotting time equivalent to that of the 80 per cent-prothrombin mixture; here the Daboia venom appeared to be equal in effect to 60 per cent of the normal prothrombin content of the plasma. The same amount of venom, however, in the presence of 0.25 per cent of the normal prothrombin, did not produce the effect of even 20 per cent of the normal prothrombin.

TABLE 5

Coagulant action of Daboia venom on plasmas with varied prothrombin concentration

PROTHROMBIN CONCENTRATION	CLOTTING TIME ON ADDITION OF $CaCl_2$ AND:	
	Optimal tissue extract (prothrombin time)	0.01 mgm. Daboia venom + optimal tissue extract
per cent	*seconds*	*seconds*
100	41	18
80	42	21
60	56	24
40	79	30
20	110	43
±0.25	*	420

* Incomplete clotting in 20 to 30 minutes.

TABLE 6

Coagulant action of equivalent quantities of Daboia venom and of tissue extract on recalcified Berkefeld-filtered bovine plasma

UNITS	CLOTTING TIME	
	Daboia venom, 1 unit = 0.001 mgm.	T. G., 297, 1 unit = 0.4 mgm.
	seconds	*seconds*
1	211	179
3	123	130
5	108	100
7	93	93
10	88	85

Quantitative effect of Daboia venom on coagulation rate. Dilutions of tissue extract and of Daboia venom having equivalent potencies were prepared, and multiples of these equivalent units of each substance were compared for their power to clot recalcified Berkefeld-filtered bovine plasma. A typical series of determinations is given in table 6. In this and similar experiments it was observed that the amount of Daboia venom added to plasma bore a roughly quantitative relationship to the coagulation rate

produced and, furthermore, that the results obtained were parallel to those observed when equivalent amounts of tissue extract were used. A mathematical statement of the relationship between tissue extract concentration and clotting time has been formulated by Mills (20) for the plasma-calcium-thromboplastin system. The data so far obtained for Daboia venom indicate a similar relationship.

Synergistic action of Daboia venom and thromboplastin. Although, as indicated above, given increments of either Daboia venom or tissue extract alone produced a proportionate acceleration of coagulation, this did not hold true when the two coagulants were employed in combination. The combined action of Daboia venom and tissue extract was not additive; it was synergistic. Typical results are presented in table 7. There it is apparent that the use of equivalent unit quantities (0.01 mgm. and 0.04 mgm. respectively) of Daboia venom and T. G. 294 in combination yielded a coagulation rate very much faster than that obtained by doubling the unit quantity of either coagulant alone.

TABLE 7

Coagulant action of equivalent amounts of tissue extract and of Daboia venom employed separately and in combination; recalcified Berkefeld-filtered bovine plasma

COAGULANT	CLOTTING TIME
	seconds
0.01 mgm. Daboia venom	82
0.04 mgm. T. G., 294	86
0.02 mgm. Daboia venom	71
0.08 mgm. T. G., 294	73
0.01 mgm. Daboia venom + 0.04 mgm. T. G., 294	30

The synergistic effect described above was observed when Daboia venom was used in combination with other thromboplastic substances (see table 8). Of particular interest was the finding that the synergistic interaction of the venom with purified cephalin was fully as marked as that observed with crude tissue extracts. That this did not represent a non-specific group reaction with phospholipids was shown by testing the interaction of the venom with lecithin. The very slight acceleration of coagulation observed on employing 4 mgm. of lecithin—comparable to that obtained with 0.002 mgm. of cephalin—may properly be ascribed to the unavoidable residual contamination of the lecithin with cephalin.

Interaction of Daboia venom and partially inactivated thromboplastic substances. It was found that the coagulant activity of certain tissue extract preparations could be partially destroyed by heat or by extraction with ether. Used in conjunction with Daboia venom, however, these extracts exhibited an activity nearly equal to that of the untreated extracts used with venom. A 2 year-old cephalin preparation, showing very little co-

agulant activity when used alone, likewise became quite as active as a fresh potent preparation when used in conjunction with the venom (see table 9).

TABLE 8

Synergistic action of Daboia venom used in combination with thromboplastic coagulants (tissue extracts, platelets, phospholipids, commercial coagulant extracts); recalcified Berkefeld-filtered bovine plasma

THROMBOPLASTIC COAGULANT		CLOTTING TIME		
Kind	Amount	Thromboplastic coagulant alone	Daboia venom (0.01 mgm.) alone*	Daboia venom + thromboplastic coagulant
		seconds	*seconds*	*seconds*
Cephalin	2 mgm.	510	84	15
Cephalin	0.002 mgm.		84	58
Lecithin	4 mgm.		84	61
Bovine platelets	5×10^8	360	60	26
Bovine platelets	7.5×10^6		63	47
Placental coagulant	2 mgm.	270	85	25
Fibrogen—Merrell	0.1 cc.	46	95	12
Thromboplastin—Squibb	0.1 cc.	1,200	165	14
Coagulen—Ciba	0.1 cc.	None in 75 minutes	165	19
Neo-Hemoplastin—Parke, Davis	0.1 cc.	None in 75 minutes	165	120

* Differences in coagulation time observed with Daboia venom alone are due to variations in different lots of plasma.

TABLE 9

Interaction of Daboia venom with fresh and with partially inactivated thromboplastic coagulants; recalcified Berkefeld-filtered bovine plasma

THROMBOPLASTIC COAGULANT		CLOTTING TIME ON ADDITION OF:	
Kind	Condition	Thromboplastic coagulant alone	Thromboplastic coagulant + 0.01 mgm. Daboia venom
		seconds	*seconds*
T. G., 294	Fresh	44	21
T. G., 294	Heated to 60°C., 1 hour	75	22
T. G., 449	Fresh	270	25
T. G., 449	Extracted with ether	1,400	31
Cephalin, Lot B	Fresh	360	16
Cephalin, Lot A	2 years old	1,800	17

Discussion. It has been shown that Daboia venom, in the presence of a sufficient concentration of calcium ions, is a highly potent coagulant with

an activity comparable to that of cephalin or tissue extract. Since this potent activity is dependent upon the presence of calcium, the action of the venom is distinct from that which has been demonstrated for papain or trypsin (21) and for the snake venoms which have been shown (11) to resemble those enzymes in their coagulant action. Furthermore, it has been shown above that the action of the venom does not depend on the presence of formed platelets, nor does the venom function as a prothrombin substitute. Moreover, it has been found that the venom, used in conjunction with any one of a variety of thromboplastic substances, exerts a synergistic action on clotting rate which has no parallel in the interactions of thromboplastic substances themselves. This finding is borne out by the data in table 3, where it it seen that the activity of tissue extract is not affected by the presence of platelets, whereas the contrary is true of the venom. Thus the venom cannot be regarded as identical with thromboplastin in its action.

The observations presented above entirely support the conclusion of Houssay and Sordelli (4) that the venom "facilitates the formation of thrombin." While the nature of this facilitating action is as yet undetermined, the data presented indicate that it consists of an "activation," or acceleration, of the function of thromboplastic substances in the conversion of prothrombin to thrombin. The interaction of the venom with cephalin is of particular significance, since cephalin is the only known chemical entity found in most (if not all) thromboplastic substances which manifests the coagulant activity of the parent substance, and is therefore widely believed to be the active principle of such substances. It may be suggested, therefore, that the coagulant action of Daboia venom involves a chemical or physical interaction with cephalin through which the interaction of cephalin with prothrombin is in turn facilitated.

SUMMARY

1. Daboia venom exerts a significant coagulant action on plasma only in the presence of a sufficient concentration of calcium ions.

2. The coagulant action of Daboia venom is independent of the presence of formed platelets.

3. The coagulant action of Daboia venom is synergistic with the action of tissue extracts, of platelets, and of cephalin.

4. In the presence of Daboia venom, the coagulant activity of certain partially inactivated thromboplastic substances is largely or wholly restored.

5. The coagulant mechanism of Daboia venom is distinct from that of thrombin, prothrombin, or thromboplastic substances and from trypsin, papain, or the snake venoms which have been shown to resemble those enzymes in their coagulant action.

6. The coagulant action of Daboia venom appears to be exerted through an interaction with tissue extracts or with the cephalin contained in such extracts.

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[3]Since this paper was submitted for publication, it has been learned that J. W. Trevan and R. G. McFarlane (Annual Report of the Medical Research Council, 1936–37, p. 143) observed an augmentation of the coagulant action of Daboia venom on mixing it with lecithin; and that others have confirmed this observation, including F. C. G. Hobson and L. J. Witts (Jour. Path. and Bact., 52, 367, 1941) who have also demonstrated quantitatively that the action of the venom is dependent on the presence of calcium, and have indicated the influence of the presence of platelets.

THE EFFECT OF ADRENALECTOMY ON FAT ABSORPTION[1]

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The study of intestinal absorption has always presented one of the most interesting problems in physiology. Not only are the pancreas, the liver and the intestinal mucosa involved but also there is considerable evidence that the adrenal cortex may play a rôle.

The influence of the adrenals on fat absorption has been supported by the experiments of Verzar and Laszt (1935), who also postulated a somewhat similar mechanism for carbohydrate absorption (Judovits and Verzar, 1937). In an extensive series of investigations, these workers found that fat absorption was inhibited by adrenalectomy in rats and that the normal function could be restored by the administration of cortical extract.

In the absence of the adrenal cortex this effect was attributed to the failure of phosphorylation, which phenomenon is postulated as a prerequisite for the absorption of the fatty acid portion of the fat molecule. The results of Artom and Peretti (1935) using iodized fats and of Sinclair (1936) who employed elaidic acid indicate that the ingested fatty acids do become incorporated into the phospholipids of the intestinal mucosa. Not only would it appear that the adrenal glands regulate fat and carbohydrate absorption but the work of Clark (1939) and of Stein and Wertheimer (1941) would seem to indicate that this gland also controls the absorption of sodium chloride.

On the other hand, evidence from this laboratory (Deuel, Hallman, Murray and Samuels, 1937) indicates that the rôle of the adrenal cortex in carbohydrate absorption is a secondary one. The lowered absorption of glucose did not occur if dehydration and the consequent circulatory disturbances were avoided by administration of Rubin-Krick or sodium chloride solution following adrenalectomy. That a similar explanation for the lowering of fat absorption in adrenalectomized animals may obtain, is indicated by the report of Barnes, MacKay, Wick and Carne (1939) who found

[1] These data are from a thesis to be presented by Lucien Bavetta to the Graduate School of the University of Southern California in partial fulfilment for the degree of Doctor of Philosophy.

no effect on the rate of absorption of methyl esters of fatty acids or of corn oil itself in adrenalectomized rats and also by that of Barnes, Miller and Burr (1939) who used spectroscopically active fatty acids.

Because of these divergent results it seemed desirable to reinvestigate the effects of adrenalectomy on the absorption of fats. In this study a comparison has been made of the rate of absorption of hydrogenated cottonseed oil in normal, sham-operated, and adrenalectomized rats which were given either Rubin-Krick solution alone, water, or water and cortin.

METHODS. The experimental animals were female albino rats from our stock colony weighing 120 to 160 grams kept on our regular stock diet. They were adrenalectomized under ether anesthesia using the lumbar approach. Experiments on fat absorption were made 7 to 10 days after the operation. In most cases the completeness of operation was checked post mortem.

The procedure for the determination of the rate of fat absorption was similar to that employed earlier (Deuel, Hallman and Leonard, 1940) and the physical and chemical constants of the fat were identical with those of the hydrogenated cottonseed oil reported in our earlier work. A fasting period of 24 hours preceded the tests. The fat was administered at a level of 300 mgm. per 100 sq. cm. of body surface. The latter was calculated by the formula of Lee (1929). During the tests the animals were kept in separate cages and any experiments showing evidence of diarrhea were discarded. The fatty acids recovered from the gastro-intestinal contents were estimated by titration of the ether extract with 0.1 N NaOH using phenolphthalein after solution in petroleum ether and isopropyl alcohol. The milligrams of fatty acids were calculated from the titration by use of a fatty acid equivalent based on the saponification number of the fat.

Cortin[2] was administered in the drinking water for 4 days prior to the absorption tests in amounts of 1 cc. daily. This amount of hormone was added each day to approximately the volume of water taken by the rat the previous day. One hour before the fat feeding, each animal was given an additional 0.5 cc. of cortin by stomach tube.

RESULTS. A summary table showing the rate of absorption of normal and adrenalectomized rats is recorded in table 1 while the control tests on the fasted animals are given in table 2.

The fat absorbed is calculated from the difference between the amount fed and the corrected amount recovered from the gut. The quantity actually recovered from the gut is first corrected by subtraction of the amount of ether-soluble material which was removable from the gastro-intestinal tracts of rats fasted for a similar period but fed no fat (table 2). A further correction is applied for the extent of recovery based on the values obtained

[2] Cortin was kindly furnished for these studies by Dr. E. C. Kendall of the Mayo Clinic who suggested the dosage and method of administration.

when known amounts of fat are given and the gastro-intestinal tract removed immediately. The latter value employed was for a 93.6 per cent recovery.

TABLE 1

Summary table showing the fat absorbed in three hour period by female rats fasted one day and fed 300 mgm. of hydrogenated cottonseed oil per 100 sq. cm. of surface area

EXPERIMENTAL CONDITION	NUMBER OF EXPERIMENTS	AVERAGE WEIGHT	AVERAGE SURFACE AREA	FAT ABSORBED IN MGM. PER HOUR*			TITRATION OF ETHER EXTRACT	FATTY ACID IN ETHER EXTRACT		NEUTRAL FAT HYDROLYZED PER HOUR*
				Per 100 gm.	Per 100 sq. cm. Total	Per 100 sq. cm. M.D.: S.E.M.D.†		Total	Per cent of total lipid	
		grams	*sq. cm.*				*cc. 0.1 N NaOH*	*mgm.*		*mgm. per 100 sq. cm.*
Normal (a)	17	117	219	68.2±1.8	36.3±1.0		3.0±0.3	83.3	24.5	46.2±1.5
Normal—Sham-operated (b)	16	125	227	63.3±2.3	34.9±1.0		2.4±0.3	66.5	15.6	46.0±1.7
Adrenalectomized—Rubin-Krick (c)	31	146	248	46.6±2.9	27.6±1.6	4.53(a) 3.88(b)	7.9±0.2	217.0	39.9	54.1±2.4
Adrenalectomized—Water only (d)	6	141	244	38.3±6.5	22.7±3.7	3.57(a) 3.19(b)	6.3±0.6	170.0	30.3	45.7±4.5
Adrenalectomized—Cortin (e)	20	127	229	71.7±3.7	39.7±2.1	4.25(c) 4.00(d)	1.7±0.3	47.1	12.9	47.7±2.7

* Including standard error of mean = $\sqrt{\frac{\Sigma d^2}{n}} \Big/ \sqrt{n}$

d = deviation from mean

n = number of observations

† Mean difference: standard error of mean difference. When this value exceeds 3.0 the results are considered significant. The letter in parentheses indicates groups with which comparisons are made.

TABLE 2

Summary table showing ether-soluble material in female rats fasted one day without fat feeding

EXPERIMENTAL CONDITION	NUMBER OF EXPERIMENTS	AVERAGE WEIGHT	TOTAL FAT IN GUT*	TITRATION OF ETHER EXTRACT	ETHER SOLUBLE EXTRACT AS FATTY ACID*
		grams	*mgm.*	*cc. 0.1 N NaOH*	*mgm.*
Normal	8	127	26.6±2.1	0.88	18.5±4.6
Sham-operated	14	105	34.0±3.5	0.42	11.7±1.3
Adrenalectomized—Rubin-Krick	19	161	28.0±3.6	0.53	14.6±1.8

* Including standard error of mean calculated as in table 1.

The neutral fat hydrolyzed was calculated to determine the comparative lipolytic action in the different groups. The total neutral fat hydrolyzed was the sum of the amount of fat absorbed plus the amount of the hydro-

lyzed fat left in the gut. The latter was determined by titration and expressed as neutral fat by multiplication of the titration value (corrected for fasting controls) by the saponification equivalent.

DISCUSSION. There is a decrease of approximately 38 per cent in the rate of fat absorption in the untreated adrenalectomized rats as compared with normals. When salt solution is administered to operated animals, the depression is somewhat less (24 per cent) but the difference is still highly significant from a statistical standpoint. Although the rate of absorption of normal rats was slightly lower on an average after undergoing a sham operation, it was significantly higher than that of the adrenalectomized animals. That the depression is to be ascribed to the absence of the adrenal cortex is evident from the fact that absorption could be completely restored by the administration of cortin. This fact is corroborated by the observation that the intestinal lymphatics appeared practically white in the normal and cortin-treated rats killed at the height of fat absorption while this was not evident in the adrenalectomized animals to which cortin was not given.

The decreased absorption apparently results from a failure to remove fatty acid at a normal rate as evidenced by greater accumulation of free fatty acids in the gut contents of the adrenalectomized animals not receiving cortin. On the other hand, there is no evidence of any decrease in lipolytic activity associated with the removal of the adrenal gland. These results would seem to support the theory of Verzar and Laszt (1935), although they do not give any evidence as to whether phosphorylation is involved.

SUMMARY

A definite inhibition in fat absorption was noted in adrenalectomized rats. It was only slightly improved by the administration of Rubin-Krick solution. After adrenalectomy larger amounts of fatty acids accumulated in the intestine than occurred normally. Both of these phenomena were restored to normal by the administration of cortin.

There is no evidence that adrenalectomy alters lipolytic activity.

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FACTORS INFLUENCING THE EXCRETION OF UROGASTRONE[1]

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The fact that urogastrone (1) extracted from urine, and enterogastrone (2) extracted from the mucosa of the small intestine, exert somewhat similar inhibitory effects on gastric secretion and motility, suggested the possibility that urogastrone may represent enterogastrone, which has been eliminated from the body by the kidneys. It is known that enterogastrone is formed by the mucosa of the small intestine and that ingestion of neutral fat is especially effective in causing its liberation (3). Accordingly, if urogastrone is the same as enterogastrone, the feeding of fat should augment its output in the urine and the removal of the small intestine should eliminate it from the urine. Neither duodenectomy nor gastrectomy eliminates urogastrone from the urine, according to Friedman *et al.* (4), but this evidence is incomplete, since enterogastrone is formed by the entire small intestine and not by the stomach. It was the purpose of the present investigation to determine the effects of complete enterectomy and the feeding of fat on the output of urogastrone. In addition, the output in patients with peptic ulcer was investigated, since Friedman *et al.* (5) state that active extracts can be prepared from the urine of ulcer patients, whereas Necheles (6) has claimed that ulcer urine contains less activity than normal urine.

METHODS. The general plan of the experiments was as follows: In order to determine the effect of removal of the small intestine, the output of urogastrone during fasting was compared with the output after complete enterectomy. The results were controlled by similarly comparing the output before and after a control or "dummy" operation. In order to determine the effect of feeding fat, the output of urogastrone during fasting was compared with the output when a high fat diet was fed. The results were controlled by similarly comparing the output during fasting with the output when a low fat diet was fed.

A total of eleven series of experiments was performed, each of which included from 3 to 5 dogs. In each series the urine was pooled for purposes of extraction. In most of the series the urine was collected entirely by

[1] Aided in part by a grant from the Committee on Endocrinology of the National Research Council.

frequent catheterization, employing female dogs previously prepared surgically for this procedure.

Fasting urine was not collected until the dogs had been without food for two days, and was continued for approximately a week. In some series the animals were given fluid only parenterally; in others they were allowed to drink water *ad lib.*

The surgical procedures were as follows. *The enterectomy consisted of* a, removal of the small intestine from the pyloric sphincter to the cecum; b, external drainage of the stomach through a large Pezzer catheter sutured into the pyloric opening; c, ligation of the common bile duct, with external drainage of the gall bladder through a small Pezzer catheter, and d, removal of the body of the pancreas leaving the ligated head and tail. The enteroantrectomy was done similarly, except that the pyloric antrum was also removed. *The control or "dummy" operation consisted of* a, transection at the incisura angularis with blind closure of the distal stump; b, external gastrostomy as above; c, ligation of the common bile duct and cholecystostomy as above; d, ligation of the pancreatic ducts. This operation differed from the others only in that the small intestine, with digestive secretions excluded, was allowed to remain in the abdominal cavity.

Beginning approximately 12 hours after the operation, urine, bile and gastric juice were collected and fluid was administered subcutaneously at intervals throughout the day and night, for a period of approximately a week. The animals remained in good condition during this interval; 6 animals were maintained for 14 to 33 days by the intravenous administration of glucose and amino acids after urine collection was terminated.

Urine was collected from dogs over a period of a week or more, during which they were fed twice daily a high fat diet consisting of prepared dog food with 30 per cent added fat, or a low-fat diet consisting of the prepared dog food alone, which contained 2.5 per cent fat.

The effect of feeding a fatty meal was also investigated in 28 human subjects. Urine was collected on one fasting day and on another day when three meals of 30 per cent cream and crackers were taken. The body weight and the exact period of urine collection were recorded for each subject and the urine was pooled for purposes of extraction.

Urine was collected similarly from eight patients with duodenal ulcer over a period of several days while they were maintained on a milk diet.

The daily urine samples were extracted by the benzoic acid adsorption method previously described (1). The crude concentrates thus obtained were further purified by various methods. As new methods of purification were developed, they were applied to the dog urines. Consequently different methods were used in the different series, although without exception the same procedure was employed within any given series.

The extracts were assayed in either Heidenhain pouch dogs, or dogs with

pouches of the entire stomach (vagotomized). In these animals the milligrams of free acid secreted in response to histamine in the forenoon was compared with the response to the same dose of histamine given 3 hours later and 10 minutes after the intravenous injection of the extract to be assayed. From a previously established curve the average percentage inhibition was converted into "doses" of urogastrone. A "dose" is defined as the quantity which produces 50 per cent inhibition under the conditions of the assay. In order to avoid the implication of a high degree of accuracy, the term "dose" is used instead of "unit."

TABLE 1

The effect of surgical procedures on the output of urogastrone

SERIES	NUMBER OF DOGS	CONDITION	ASSAY: Number of assays	ASSAY: Dose: Mgm.	ASSAY: Dose: Cc. urine	ASSAY: Dose: Kgm.-hrs.	ASSAY: Effect: Temp. rise	ASSAY: Effect: % inhib.	ASSAY: Effect: Inhib. doses	YIELDS: Per 100 cc. urine: Extr.	YIELDS: Per 100 cc. urine: "Doses"	YIELDS: Per 100 kgm. hrs.: Urine	YIELDS: Per 100 kgm. hrs.: Extr.	YIELDS: Per 100 kgm. hrs.: "Doses"	YIELDS: Per 100 kgm. hrs.: % change
							°F.			mgm.		cc.	mgm.		
1	3	Fasted + fluid	17		350	105	0.75	−34	0.76		0.22	360		0.72	
		Enterectomy	14		555	150	1.10	−31	0.66		0.12	336		0.44	−39
2	4	Fasted + fluid	12	5	393	205	1.35	−32	0.70	1.27	0.19	191	2.46	0.34	
		Enteroantrectomy	11	6	209	209	1.05	−14	0.26	2.81	0.12	100	2.81	0.12	−65
			12	9	188	207	1.25	−8	0.14	4.80	0.07	91	4.35	0.07	−80
3	4	Fasted	9	7	216	505		−44	1.08	3.24	0.49	43	1.38	0.21	
		Enterectomy	5	20	301	506		−40	0.94	6.65	0.30	60	4.00	0.19	−10
4	5	Enteroantrectomy	12	20	474	504	0.90	−30	0.64	4.22	0.14	94	3.96	0.13	−38*
1	4	Fasted + fluid	12	4	203	277	0.65	−35	0.80	1.87	0.39	77	1.44	0.29	
		Control operatn.	18	15	135	195	1.30	−53	1.40	11.13	1.00	70	7.76	0.72	+148
2	4	Fasted	7	5	167	391		−36	0.82	3.00	0.50	44	1.34	0.21	
		Control operatn.	10	13	273	308	0.20	−36	0.82	4.77	0.31	89	4.22	0.27	+29

* Calculated assuming lowest fasting output in any of other series, namely, 0.21 "doses" per 100 kgm. hrs.

The rectal temperature of the assay animals was taken at half-hour intervals after the injection of the extracts until the maximal rise was recorded. In the case of dog urine extracts we have not been able to eliminate regularly all traces of pyrogenic substances.

RESULTS. *The effects of surgical procedures.* The essential data concerning the 6 series of experiments are presented in table 1. The doses of extract administered to the assay animals are recorded in 3 ways; as milligrams and as the volume of urine, and kilogram hours represented by the milligram dose. The yields by weight of extract and of "doses" of urogas-

trone per 100 cc. of urine are included, although these do not represent a true measure of output. In measuring the urinary output of a substance, both the weight of the dog and the period of excretion must be considered. Hence, the output of urogastrone is obtained from the column headed, yield of "doses" of urogastrone per 100 kgm. hrs. Since, as will be pointed out later, the rate of urine production affects the recovery of urogastrone, the former is recorded as the volume of urine secreted per 100 kgm. hrs. The last column records the most significant figure, namely, the percentage change in urogastrone output produced by the various procedures. Since the assay dogs, the method of handling the operated animals, the extraction procedures were constant *within* any given series, but not necessarily *between* different series, it may be misleading to make any comparison other than that shown in the last column.

As shown in table 1, the removal of the entire small intestine (with or without antrectomy) reduced the output of urogastrone by 10 per cent to 80 per cent in the 4 series of experiments. A fifth series, not included in the table, showed the usual low post-operative output of urogastrone, but for an unidentified reason the extract of the fasting or pre-operative urine was completely inactive. It will be noted that in series 2 the output was reduced 65 per cent during the first two post-operative days and 80 per cent during the subsequent 3-day period.

In contrast to the above results, the output of urogastrone was increased 29 per cent and 148 per cent in the two control series in which the "dummy" operation, consisting of the exclusion of digestive secretions from the intestine, was performed.

It should be noted that following either type of operation the yields *by weight* of extract are greatly increased. This necessitated the use of large milligram doses to correspond to the pre-operative kgm.-hr. doses. The bearing of this fact on the interpretation of the results will be discussed later.

The effect of diet. The essential data concerning the 4 series of experiments are presented in table 2. In 3 series of experiments, 1 in human subjects and 2 in dogs, the feeding of a high fat diet increased the output of urogastrone 99 per cent, 23 per cent, and 194 per cent over the fasting output. However, the ingestion of a practically fat-free diet similarly increased the output 86 per cent in dogs.

The effect of peptic ulcer. Table 2 also includes the results obtained with the extracts prepared from the urine of ulcer patients. The urogastrone output was found to be 0.037 dose per 100 kgm. hrs. in these patients receiving a milk diet in contrast to the higher outputs of 0.077 and 0.153 dose respectively for normal subjects, fasting, or receiving a cream diet. The increased urine volume of the ulcer patients yielded more extract, but its potency was so reduced as to diminish significantly the output of urogastrone activity.

The effect of urine production. In the course of the above experiments it was noted that the rate of urine production influenced the apparent fasting output of urogastrone in dogs. In table 3 the yields of urogastrone "doses" per 100 kgm. hrs. and also the yields per 100 cc. of urine are presented together with the volume of urine excreted per 100 kgm. hrs. It

TABLE 2

Urogastrone outputs in fasted and fed subjects, and ulcer patients

SERIES	NUMBER OF DOGS	CONDITION	ASSAY							YIELDS					
			Number of assays	Dose			Effect			Per 100 cc. urine		Per 100 kgm. hrs.			
				Mgm.	Cc. urine	Kgm.-hrs.	Temp. rise	% inhib.	Inhib. doses	Extr.	"Doses"	Urine	Extr.	"Doses"	% change
							°F.			mgm.		cc.	mgm.		
1	4	Fasted + fluid	6	6	396	206	2.7	−47	1.18	1.51	0.298	191	2.89	0.57	
		High-fat diet	11	4	220	162	1.7	−46	1.14	1.82	0.518	135	2.46	0.70	+23
2	4	Fasted + fluid	12	5	393	205	1.3	−32	0.70	1.28	0.178	191	2.43	0.34	
		High-fat diet	11	5	235	128	1.1	−50	1.28	2.19	0.545	178	3.91	1.00	+194
3	4	Fasted	8	3	378	579	0.9	−57	1.62	0.80	0.428	66	0.518	0.28	
		Low-fat diet	9	3	478	321	0.9	−58	1.68	0.63	0.352	149	0.935	0.52	+86
	MEN														
4	28	Fasted	15	5	1235	2185	0.7	−58	1.68	0.41	0.136	56	0.229	0.077	
		Cream diet	12	5	1130	1565	0.2	−69	2.40	0.44	0.213	71	0.319	0.153	+99
5	8	Ulcer patients, milk diet	15	10	2196	1900	0.6	−32	0.70	0.46	0.032	115	0.526	0.037	

TABLE 3

Effect of urine flow on extract yields

URINE FLOW PER 100 KGM. HRS.	CRUDE EXTRACT		INHIBITORY ACTIVITY	
	Per 100 cc.	Per 100 kgm. hrs.	Per 100 cc.	Per 100 kgm. hrs.
cc.	*mgm.*	*mgm.*	*"doses"*	*"doses"*
44	40.3	17.8	0.50	0.21
66	28.1	18.4	0.43	0.28
77	40.2	30.9	0.39	0.29
191	28.5	54.5	0.24	0.45
360	20.3	73.0	0.22	0.72

can be seen that as the rate of urine excretion increases, the output of urogastrone also increases, but not at the same rate, since the concentration of urogastrone in the dilute urine is reduced. Similar relationships are obtained if the weight of crude extract is substituted for "doses" of urogastrone. It should be recalled that the curves are influenced to an unknown

extent by the variations in the procedures employed in the different fasting series.

It has been found that the degree of recovery of solid material from urine by the benzoic acid procedure may be in part responsible for the above results. For example, one liter of pooled human urine yielded 60 mgm. of crude concentrate; another liter from the same pooled specimen when diluted with an equal quantity of water and then extracted as two liters of urine, yielded 75 mgm. of crude concentrate. Hence with a constant total quantity of solids, the more dilute it is the greater the total recovery, but the less is the yield per unit volume. Accordingly, the non-quantitative nature of the extraction procedure may be responsible for the apparent influence of diuresis on the output of urogastrone.

If tables 1 and 2 are re-examined in the light of the above findings, it will be seen that variations in the rate of urine production do not account for the effect of surgical procedures or of diet on the output of urogastrone.

Discussion. If the results obtained are to be accepted at face value, they indicate that the output of urogastrone is reduced by removal of the small intestine, and increased by the induction of diuresis, by the ingestion of a high or low fat diet, and by the exclusion of digestive secretions from the small intestine. However, evidence was obtained which suggested that the apparent effect of diuresis may be due to an improved recovery of urogastrone from the urine rather than to an increased output. Furthermore, it must be remembered that the methods of extraction are not strictly quantitative, and that neither the assay nor extraction procedures are specific for a single biologically active compound. Hence, one must be cautious in the interpretation of the results.

The extent to which these deficiencies in methods may influence the final interpretation of the results is considerable. For example, more material was extracted from the post-operative than from the preoperative urines, which necessitated the use of larger doses for assay. Since the extraction procedure is not quantitative, this greater yield could be indicative of a more complete recovery of urogastrone from the post-operative urines. If this were actually the case, the effect of enterectomy should properly be revealed by a comparison of the outputs after enterectomy with the outputs after the control operation; this would imply a marked reduction in urogastrone output following removal of the small intestine. On the other hand, since the methods are not specific, it could be maintained that the extra material extracted from the post-operative urines, which probably consists of products of autolysis of the surgically traumatized tissue (8) might produce a "non-specific" inhibition. If this were actually the case, the *extra* activity after the control operation and the *entire* activity after enterectomy could be attributed to these "non-specific" inhibitor substances; this would imply that enterectomy completely eliminates urogastrone from the urine.

Although the above interpretations cannot summarily be dismissed from consideration, neither can they be accepted to the exclusion of other interpretations. In short, the results do not permit a final decision regarding the effect of enterectomy on the excretion of urogastrone. What can be said with a reasonable degree of certainty is that *a*, the exclusion of digestive juices from the small intestine (control operation) does not decrease the output of inhibitor substance or substances, and *b*, if there is only one inhibitor substance in the urine extracts, it does not originate entirely from the small intestine.

It is conceivable that enterogastrone is liberated in small quantities under fasting conditions and in larger quantities during the digestion of a low-fat meal, although there is no direct evidence bearing on these points (7). Accordingly, in these respects the behavior of urogastrone is not incompatible with the view that it represents excreted enterogastrone. However, the ingestion of fat should be the most effective stimulus for the release of enterogastrone and in this respect urogastrone appears not to behave like enterogastrone. This evidence, in conjunction with the uncertain results of the enterectomy experiments, makes the weight of evidence favor the view that urogastrone and enterogastrone are separate entities. On the other hand, the fact that enterectomy reduces, feeding augments, and the presence of a duodenal ulcer reduces the output of urogastrone, points to the importance of the gastro-intestinal tract for the control of urogastrone excretion.

CONCLUSIONS

1. The apparent excretion of urogastrone is decreased by removal of the small intestine and increased by the induction of diuresis, by the ingestion of a high or low fat diet, and by the exclusion of digestive secretions from the small intestine (control operation).

2. Since the apparent excretion may not truly represent the actual excretion, for reasons discussed, the acceptable conclusions are limited to the following:

a. The exclusion of digestive secretions from the small intestine (control operation) does not reduce the output of inhibitor substance(s).

b. If only one inhibitor substance is present in the urine extracts, it does not originate entirely from the small intestine.

c. A high-fat diet is no more effective than a low-fat diet in augmenting the output of inhibitor substance(s).

3. The output of urogastrone is reduced below normal in patients with peptic ulcer.

4. The evidence suggests the importance of the gastro-intestinal tract for the regulation of urogastrone excretion, but does not support the view that urogastrone and enterogastrone are identical.

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THYROID ACTIVITY AFTER IODINE INGESTION

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In hyperthyroid patients iodine is commonly given to depress the activity of the thyroid in preparation for an operation. In animals iodine has been shown to diminish the responses of the thyroid which are ordinarily produced by giving anterior pituitary extracts (see Siebert and Linton, 1935; Friedgood, 1936; Cutting and Robson, 1939). In order to learn more about this effect, we have studied rats during prolonged exposure to cold. The elevation of metabolism in this case is due to the release of extra hormone from the thyroid (see Ring, 1939). The increase occurs in the absence of the cervical sympathetics and is probably brought about by the anterior pituitary (Ring, 1939; Uotila, 1939). Our problem was to find out whether the metabolic response to cold could be depressed by the ingestion of iodine. First, we decided to see whether iodine affected the normal basal metabolism of rats. Only when rats were given doses of iodine so large that they lost weight, did the metabolism fall. The withdrawal of this extra iodine, however, brought about a much more decided reduction in metabolism which lasted for several weeks. We have followed this change in some detail.

METHOD. In the first group of experiments, 20 mgm. of NaI were placed in the water which each rat was expected to drink during one day (ordinarily 25 cc.). This amount was supplied daily for one week and the basal metabolism was then measured by means of a modification of Benedict's Multiple Chamber Respiration Apparatus (see Ring, 1940). Each subsequent week, the NaI added to the drinking water was doubled until the animals started to lose weight. Then water without NaI was supplied and metabolic measurements were continued at weekly intervals until they approached normal.

A second procedure consisted in giving rats drinking water which contained 0.75 mgm. of NaI per cc. Measurements of basal metabolism were made at weekly intervals for 3 weeks. These rats weighing about 200 grams each ingested 20 to 25 mgm. NaI per day. This did not affect their basal metabolism nor stop their growth. These and similar rats were then divided into 5 groups. Group A was placed in the refrigerator at 2° to 4°C. for 3 weeks and supplied with drinking water containing NaI. Group B

was given ordinary drinking water while in the refrigerator. All of the animals in group B died before measurements of metabolism were made. Therefore, in group C, the NaI was discontinued for five days before as well as during their stay in the refrigerator. Most of this group survived. Groups D and E, after the withdrawal of NaI were given 25 guinea pig units of anterior pituitary thyrotropic principle[1] per 100 grams of body weight each day for four days. Group D was then placed in the refrigerator while group E was kept at room temperature. This last group was used to study the effects of extract alone upon metabolism. Finally some normal rats were placed in the refrigerator as controls. All rats were fed on Purina Dog Chow which has been shown to contain adequate amounts of I (see Remington and Remington, 1938). This food supplied about 4 gamma of I per day when the rats were kept at room temperature and about twice that amount while they were living in the refrigerator.

RESULTS. Table 1 shows the basal metabolism of rats during and after the ingestion of NaI in amounts large enough to stop growth. The quantity which each rat would tolerate was quite variable. The substitution of water for NaI solution permitted growth to begin again but the metabolism fell and remained subnormal for weeks. When one large dose of NaI (1200 mgm.) was given, the results were similar. The control measurements on six of these rats averaged 780 cal. per sq. m. per day. After ingesting NaI, the metabolism, measured at weekly intervals, averaged 705, 718, 724, 743. Of course the giving of such large amounts of NaI leads to a temporary loss of weight, and this in itself might lower metabolism. However when inanition is produced by fasting, the metabolism returns to normal a few days after food is supplied. It is hardly likely therefore that this would account for the prolonged changes observed.

When smaller doses of NaI (20–25 mgm. daily) were given for a period of three weeks, growth continued and basal metabolism was probably not depressed. The results are shown in table 2. The fall of 2 per cent in metabolism is due, we believe, to the animals becoming more accustomed to the apparatus. As large a drop commonly occurs in untreated animals. After stopping the ingestion of this amount of NaI, other experiments have shown that there is no fall in basal metabolism. Nevertheless, the usual responses of the thyroid to stimulation could not be evoked. This was shown in two ways—by injecting Antruitrin T and by subjecting the animals to cold over a prolonged period of time. The third table indicates the changes produced by injecting thyrotropic principle. In the control rats, these injections caused an increase in metabolism reaching its maximum of 10 per cent two weeks after the injections were started. Rats that had previously received NaI did not show any metabolic change.

[1] The thyrotropic principle, antuitrin T, was kindly supplied by Parke, Davis and Company.

Of twelve rats placed in the refrigerator at the time NaI was withdrawn, none survived for more than two weeks. Apparently the thyroid could not make an appropriate response to the cold environment so that the body temperature fell and the animals died. When the NaI was withdrawn 5 days before placing the animals in the refrigerator, 7 out of 8 rats survived but their metabolic response to cold was smaller than in normal animals, as

TABLE 1

Prolonged feeding with NaI in drinking water

RAT NUMBER	CONTROL METABOLISM WITH NaI INGESTION (CAL./SQ.M./DAY)			AFTER WITHDRAWAL OF NaI							AMOUNT OF NaI GIVEN PER DAY
	1st wk.	2nd wk.	3rd wk.	4th wk.	5th wk.	6th wk.	7th wk.	8th wk.	9th wk.	10th wk.	
											mgm.
1	768	736	696	668	652	660	692				80
1	692	687	676	627	636	669	692	703			320
2	739	740	769	662	637	754	671				180
3	722	668	677	611	589	655	680				160
3	686	670		637	643	563	610	612	657	699	40
4	734	787	774	701	693	724	691	731			180
5	772	788	675	677	713	695	717	783			180
Average met.	730	725	705	655	652	674	679				
Average wt.	278	276	262	271	275	282	290				

TABLE 2

Metabolism before and during the ingestion of NaI*

	CONTROL PERIOD		GIVEN (MGM. NaI PER CC. DRINKING WATER)	DURING NaI INGESTION		
	1st wk.	2nd wk.		3rd wk.	4th wk.	5th wk.
			mgm.			
Average metabolism of 7 rats	858 ±11.0	836	0.75	840	827	817 ±12
Average weight	224.0	230.6		238.6	246.3	251.8

* In calories per square meter per day.

shown in table 4. The difference between the two groups of animals is not large but statistically there are 39 chances in 40 that this difference is significant.

Rats given NaI during their stay in the refrigerator showed an elevation in metabolism which was at least as great as that found in the controls (see table 4). It appears that when NaI is given after the rats leave the refrigerator the metabolism returns to normal more quickly. Of the control

group 7 out of 9 still showed an elevated metabolism four weeks after leaving the refrigerator whereas none of those receiving NaI showed any elevation at this time. In fact the metabolism of the latter group was slightly depressed.

If animals from which NaI has been withdrawn are given Antuitrin T and then placed in the refrigerator they show the largest elevation in metabolism of any group. This is in spite of the fact that without the cold stimulus, thyrotropic principle will not elevate the metabolism of otherwise similarly treated animals.

TABLE 3

Metabolism of rats after receiving thyrotropic principle*

(25 guinea pig units per 100 grams per day for 4 days)

	CONTROL		1ST WK.	2ND WK.	3RD WK.	4TH WK.
5 control rats..................	787	Thyrotropic	799	862	826	
6 rats after NaI ingestion.....	799	principle	772	801	787	773

* In calories per square meter per day.

TABLE 4

Change in basal metabolism after three weeks' exposure to cold (in per cent)*

CONTROLS	RATS GIVEN NaI UNTIL 5 DAYS BEFORE EXPOSURE TO COLD	RATS GIVEN NaI DURING EXPOSURE TO COLD	RATS GIVEN ANTUITRIN T AND NaI BEFORE EXPOSURE TO COLD
(Measured after being at room temperature for one day)			
+3.9	−1.5	+4.0	0
4.6	+1.0	7.1	+11.9
5.4	2.7	9.7	14.7
8.0	5.8	13.4	15.1
9.0	7.2	16.1	22.8
9.6	7.8	19.8	24.6
10.7	12.2		
13.5			
17.0			
Average.... 9.1 ±0.9	5.0 ±0.7	11.7 ±1.5	+14.9 ±2.2
(Measured after being at room temperature for one week)			
Average.... 4.6	+3.1	+7.0	+8.0

*Measured at 30°C.

Discussion. Our results clearly show that NaI will not prevent the usual elevation in metabolism produced by cold. According to Starr and Roskelley, the injection of NaI into rats kept in a refrigerator limits the

hypertrophy of the thyroid. It is therefore apparent that no correlation exists between the metabolic response and the histological picture under these circumstances.

When the stimulus due to the cold environment is removed, the metabolism returns to normal more quickly if the ingestion of NaI is continued. This response is somewhat similar to that observed when hyperthyroid patients are given I.

It is surprising that after giving large amounts of NaI, the withdrawal depresses thyroid function. Possibly the thyroid gland becomes accustomed to a certain level of I in the blood and when this level falls, the gland fails for a time to take up the iodine it needs to manufacture new hormone.

When rats stop taking NaI, thyrotropic principle will apparently prepare the thyroid to make a suitable adjustment to cold. In fact the response obtained was greater than either stimulus alone would produce. In two rats which did not receive NaI, thyrotropic principle seemed to increase the metabolic response to cold in a similar manner.

CONCLUSIONS

1. When rats, kept in a refrigerator for 3 weeks, are given 20 to 25 mgm. NaI per day, an elevation in basal metabolism occurs which is as great as that found in control rats (see table 4).
2. After being placed in a warm environment the metabolism of these rats returns to the control level more quickly if they are given NaI.
3. The ingestion of NaI does not depress the metabolism of normal rats unless the amount given is large enough to cause inanition.
4. The withdrawal of large doses of NaI (in most cases 160 mgm. per day) frequently depresses basal metabolism for several weeks thereafter (see table 1).
5. The withdrawal of smaller doses of NaI (20–25 mgm. per day) does not bring about a measurable reduction in metabolism. It does prevent the occurrence of as large an elevation of metabolism as that ordinarily produced by either thyrotropic principle or prolonged exposure to cold.
6. When thyrotropic principle is given and followed by prolonged exposure to cold, the metabolic response is greater than that produced by either stimulus alone (see tables 3 and 4).

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THE EFFECT OF VARYING RESISTANCE-LOAD AND INPUT-LOAD ON THE ENERGETICS OF THE SURVIVING MAMMALIAN HEART[1]

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The law of the heart enunciated by Starling (1) that the output of the heart is a function of its diastolic size, has been amplified to apply also to its work and energy expenditure by Starling and Visscher (2). This would indicate that the relation: work/energy expenditure, i.e., the mechanical efficiency of the heart, is also a function of the diastolic heart size. However Gollwitzer-Meier et al. (3, 4) and Gremels (5) have indicated that the mechanical efficiency of the heart is also dependent upon whether the change in size is induced by a change in the arterial resistance or by a change in the venous return. They reported that the augmentation of the work of the heart noted on increasing the arterial resistance is accompanied by a much greater increase in energy expenditure than a like augmentation of work observed on increasing the venous return to the heart. The mechanical efficiency at a given increased level of work would thus appear to be less when the work increase is due to an increased resistance-load than when it is due to an increased input-load.

Objections can be raised to the methods used by these investigators. Gollwitzer-Meier et al. (3, 4) assumed that coronary sinus blood was a representative sample of the mixed coronary venous blood as far as O_2 content is concerned, and this has been shown to be not necessarily true (6). Gremels (5) assumed that the coronary sinus flow was always 60 per cent of the total coronary flow, and this too has been shown to be erroneous (7, 8).

It was deemed advisable, therefore, to reinvestigate the subject employing a method which would measure more accurately both the total work of the heart and its total energy expenditure based upon oxygen consumption while doing this work.

In this study we utilized either the isolated heart preparation which we have described previously (9, 10) or a special heart-lung arrangement in which the circulating blood volume could be adjusted (fig. 1). The input-

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[2] Emanuel Libman Fellow.

load was altered in the former by changing the resistance to inflow from the blood reservoir, in the latter by changing the circulating blood volume. The resistance-load was altered in both preparations by adjusting the resistance in the left and right heart outflow circuits. In both preparations, when the rate of inflow was changed, the peripheral resistances were adjusted so that the systemic and pulmonary arterial pressures (the resistance-load) were kept constant. Likewise when the resistance-load was changed, the inflow or the circulating blood volume was adjusted so that the cardiac minute output (the input-load) was kept constant.

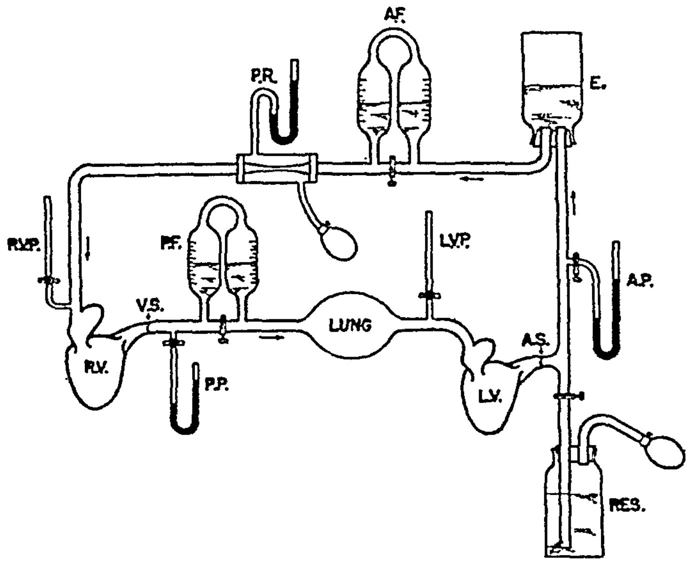

Fig. 1. Diagram of the closed-circuit heart-lung preparation. *L.V.P.* is the left venous pressure manometer; *L.V.*, the left ventricle; *A.S.*, the point where the aortic blood sample is taken; *RES*, the blood reservoir which is used to add or remove blood from the heart-lung circuit, this is controlled by the screw clamp on the tube connecting it to the heart-lung circuit and by the pressure bulb; *A.P.*, the aortic pressure manometer; *E.*, the artificial arterial elastic reservoir; *A.F.*, the aortic flowmeter; *P.R.*, the manometer measuring the degree of artificial peripheral resistance which is controlled by a pressure bottle and bulb; *R.V.P.*, the right venous pressure manometer; *R.V.*, the right ventricle; *V.S.*, the point where the pulmonary blood sample is taken; *P.P.*; the pulmonary arterial pressure manometer; and *P.F.*, the pulmonary artery flowmeter.

The work of the two ventricles was measured as in our previous studies (9, 10). Energy cost was computed as before, but the method of measuring O_2 consumption was altered to avoid the criticism applied to our previous work that the coronary sinus sample was not a true mixed venous sample. The blood samples for O_2 determination in this study were drawn under oil simultaneously from the aortic and pulmonary arterial tubes, the former giving the arterial blood, the latter the mixed venous blood. The actual O_2 content measurements were carried out as before (9) by the Van Slyke method and the A-V O_2 difference determined from the difference in O_2

content of the two samples. This multiplied by the total flow in the pulmonary artery at the time the samples were taken gave the O_2 consumption of the heart, viz:

$$O_2 \text{ consumption (cc./min.)} = \frac{\text{A-V } O_2 \text{ difference (vol. per cent)} \times \text{pulmonary arterial flow (cc./min.)}}{100}$$

This was possible because the blood in the pulmonary artery is a mixture of (1) blood that has passed through the heart cavities without the loss of oxygen, i.e., aortic blood, and (2) blood that has passed through the coronary system and has had oxygen removed from it by the heart muscle. The only cause for the difference in oxygen content between aortic and pulmonary samples is thus the O_2 utilization of the heart[3].

If P_f, A_f and C_f represent in cc./min. the flow in the pulmonary artery, aorta and coronary system respectively, and P_o, A_o and C_o represent the O_2 content of the blood in vol./100 cc. in the pulmonary artery, aorta and the true mixed coronary venous blood, respectively, then

$$P_fP_o = A_fA_o + C_fC_o$$

from which

$$C_0 = \frac{P_fP_0 - A_fA_0}{C_f}.$$

Since O_2 consumption $= C_f\,(A_o - C_o)$, it follows that

$$O_2 \text{ consumption} = C_f\left\{A_0 - \frac{(P_fP_0 - A_fA_0)}{C_f}\right\}, \text{ or}$$

$$O_2 \text{ consumption} = C_fA_o - P_fP_o + A_fA_o, \text{ or}$$
$$O_2 \text{ consumption} = (C_f + A_f)\,A_o - P_fP_o.$$

Since $P_f = C_f + A_f$,

$$O_2 \text{ consumption} = P_f\,(A_o - P_o),$$

that is, the O_2 consumption of the heart can be accurately measured by the product of pulmonary flow and A-V O_2 difference.

Four preparations were used, 3 special heart-lung preparations and 1 isolated heart preparation. Ether was the anesthetic agent employed until the blood supply to the head was interrupted. The aeration from this point until the preparation was ready for use, a half-hour at least, was sufficient to remove most, if not all, of the ether from the blood. Heparin was used as the anticoagulant in the dog from which the preparation was made and also was added to the defibrinated blood obtained for circulation. Details of preparation were carried out as previously, the only variation being

[3] The coronary venous return via Thebesians to the left heart, which is small and almost constant, estimated as 6 ± 4 per cent of the coronary flow (8), is the remaining source of error.

in the special heart-lung preparation. Here the lungs were left in situ and a blood reservoir was connected to the aortic circuit via a side arm which was kept clamped except when changes in blood volume were desired (fig. 1).

When the preparation was ready, the dynamic conditions set, and the heart stabilized to them, control blood samples, pressure, flow, peripheral resistance, heart rate and blood temperature readings were taken. The readings were repeated every 1 to 2 minutes. Two or three control blood samples were obtained at approximately 15 minute intervals. The work level was then raised by increasing either the resistance- or the input-load, the other being kept constant. Usually two more sets of blood samples were taken and then the work restored to its previous level by reducing the factor which had been increased. Another set of blood samples was taken at the reëstablished control level and the work then increased to about the same level as before, but this time by increasing the load which had previously been kept constant, the other load now being unaltered. Again, after a period of observation during which two sets of blood samples were taken, the work was reduced to the control level and a final set of blood samples taken. From these data the work, O_2 consumption and mechanical efficiency could be calculated as in our previous studies, differing only in that the new method of determining O_2 consumption, as described above, was employed.

RESULTS. The results are summarized in table 1, and illustrative experiments are shown in figures 2 and 3.

Control values. The control values in these four experiments ranged as follows:

Aortic pressure	86–101 mm. Hg
Pulmonary arterial pressure	16–27 mm. Hg
Pulmonary artery flow	98–175 cc./min.
O_2 consumption	2.7–4.0 cc./min.
Work	9–17 kgm.M/hour
Efficiency	2.2–4.3 per cent

Effect of increasing the resistance-load. As the results in table 1 show, the increase in arterial blood pressure was accompanied by a slight but consistent decrease in the average values of mechanical efficiency, that is, the O_2 consumption increased out of proportion to the increase in work. This enhancement of O_2 consumption was greater in the 1st determination after the increase in work than in the 2nd one. At the time of the 2nd determinations the efficiencies were well within the control range. We do not consider the slight average decrease in efficiency to be of significant degree although it was consistent. *There was no instance of any increase in efficiency.* During the period of increased resistance-load the coronary flow rose from 8 to 75 per cent above the control level. In two of the heart-lung

preparations the pressure in the pulmonary veins rose also. In all instances the heart increased in diastolic size on inspection.

Effect of increasing the input-load. As the results in table 1 show, the increase in venous inflow was accompanied by a definite and consistent increase in the average values of mechanical efficiency, that is the O_2 consumption did not increase in proportion to the work. In fact, in the isolated heart preparation, the 2nd determination showed an O_2 consumption lower than in the control. In the other preparations, the O_2 consumption rose somewhat, and at the time the 2nd samples were taken had returned to within the control levels.

TABLE 1

Effect of increased work on mechanical efficiency of heart

PREPARATIONS	INCREASE IN RESISTANCE LOAD					INCREASE IN INPUT-LOAD				
	Time of readings after dynamics were altered	Increase in work	Average control efficiency	Average efficiency during work increase	Change in efficiency with increase in work	Time of readings after dynamics were altered	Increase in work	Average control efficiency	Average efficiency during work increase	Change in efficiency with increase in work
	min.	*per cent*	*per cent*	*per cent*	*per cent*	*min.*	*per cent*	*per cent*	*per cent*	*per cent*
Heart-lung 1	13 and 19	50	3.2	2.6	−20	5 and 11	100	3.2	5.3	+65
						10 and 20	50	3.1	3.8	+20
Heart-lung 2	15	30	4.3	3.4	−25	6 and 13	50	3.7	7.0	+90
Heart-lung 3	9 and 14	30	3.5	3.1	−10	11 and 22	25	3.5	4.9	+40
Isolated heart	15 and 25	22	2.3	1.9	−15	18 and 24	26	2.4	3.9	+60
Range of efficiency in per cent on increasing work					−10 to −25					+20 to +90

The coronary flow did not alter appreciably except in the isolated heart preparation, where it increased during the period of increased work. The right venous pressure rose in two of the heart-lung preparations. In all instances the heart increased in diastolic size on inspection.

DISCUSSION. Our results are thus in general agreement with those of Gollwitzer-Meier and Gremels, and the objection raised to the accuracy of their work is overcome. Increasing the load of the heart increases its work, but the degree to which its oxygen consumption is increased depends also upon the nature of the increased load. Within the range employed in these experiments increasing the resistance to emptying of the heart leads to an increase in oxygen consumption in proportion to or greater than the increase in work; therefore, no increase in the mechanical efficiency of the heart occurs and, at times, a slight decrease actually appears. Similarly increasing the minute volume output of the heart within the limits studied

leads to a definitely improved mechanical efficiency; in fact, at times the O_2 consumption does not appear to increase at all.

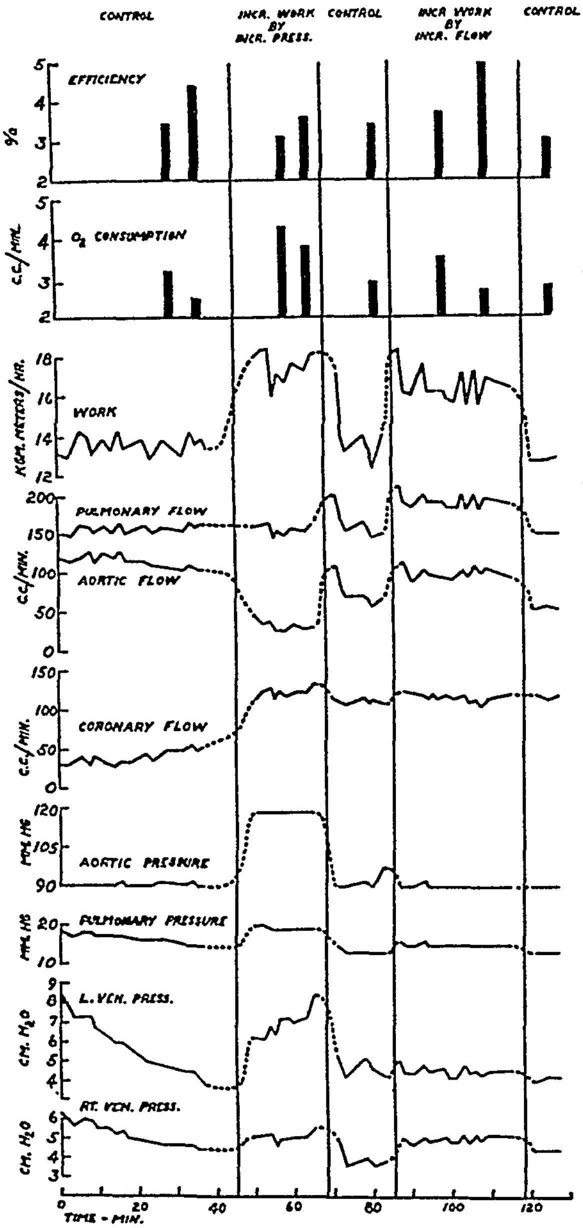

Fig. 2

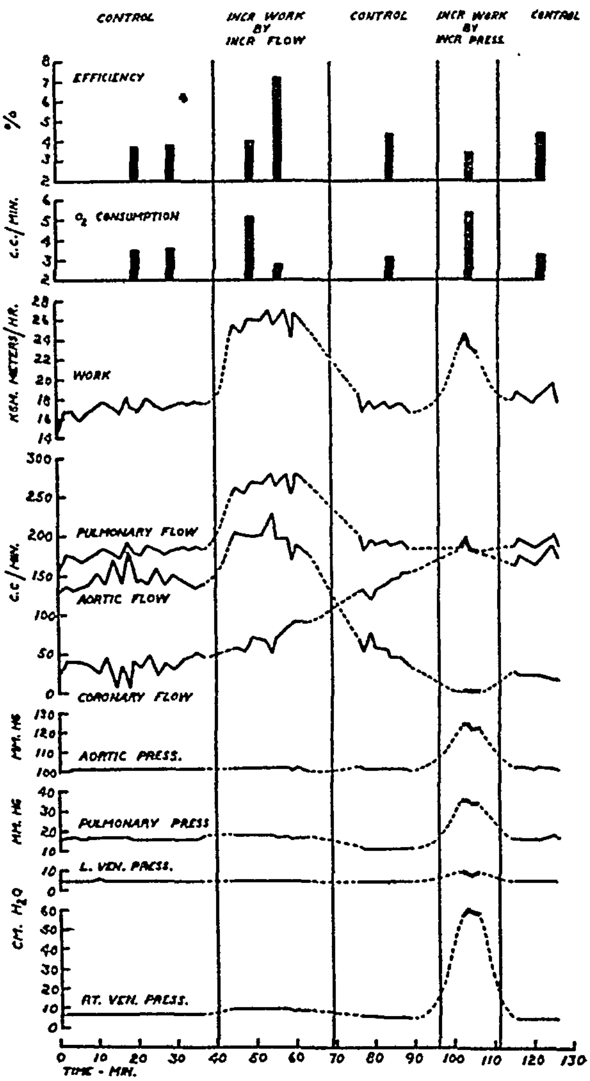

Fig. 3

Fig. 2. Chart listing pertinent data on one (heart-lung preparation) of four experiments discussed in text. *L. ven. pressure* and *Rt. ven. pressure* are left and right venous pressures. *Incr.* equals increased. Dotted lines are used to connect periods where no observations were made.

Fig. 3. Chart listing pertinent data on another experiment (heart-lung preparation) discussed in text. Conventions as in figure 3.

These results therefore cannot be taken to confirm the concept in the surviving heart that the increase in heart size which is associated with increase

in load is always accompanied by an increase in mechanical efficiency as has been assumed in the past. Both methods of increasing the work of the heart, namely, by increase of resistance-load and by an increase of input-load, are accompanied by an increase in heart size clearly seen on inspection and yet the results as far as mechanical efficiency is concerned are different. This can be shown by plotting work against O_2 consumption. Two such graphs are shown in figures 4 and 5. In each it will be seen that while increase in work is accompanied by a tendency for the O_2 consumption to increase, the increase in the latter is much greater when the resistance-load is responsible for the increased work than when this is due to an increased input-load. The lines connecting the control values with those of the in-

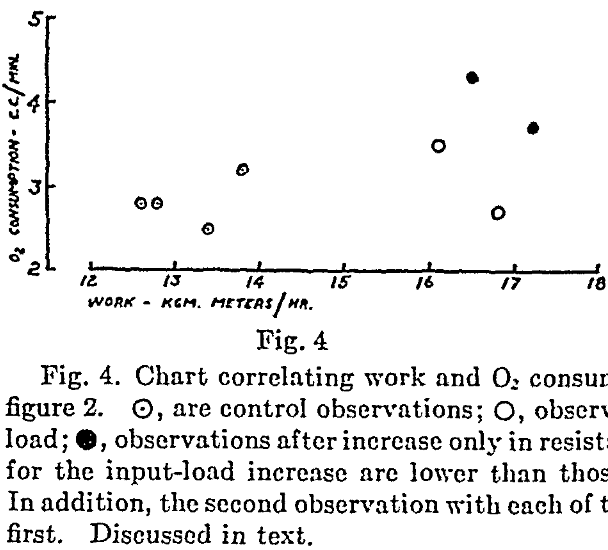

Fig. 4

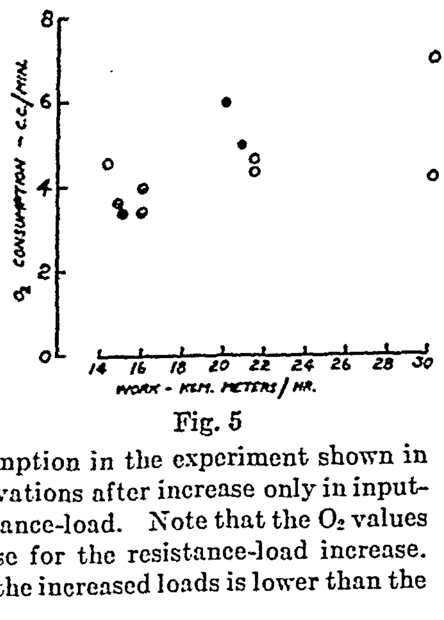

Fig. 5

Fig. 4. Chart correlating work and O_2 consumption in the experiment shown in figure 2. ⊙, are control observations; ○, observations after increase only in input-load; ●, observations after increase only in resistance-load. Note that the O_2 values for the input-load increase are lower than those for the resistance-load increase. In addition, the second observation with each of the increased loads is lower than the first. Discussed in text.

Fig. 5. Chart correlating work and O_2 consumption in another experiment. Conventions as in figure 4. The changes noted in figure 4 are shown here also. Discussed in text.

creased work under the two conditions do not coincide as would be demanded if heart size were the only factor involved. It follows therefore that other factors in addition to heart size determine the efficacy with which the heart utilizes its energy for its work.

The possible objection that the difference between these two types of increased load is due to an increase in the kinetic energy factor is not valid since under the conditions of the experiments the latter is of the order of $\frac{1}{500}$ of the potential energy used to calculate the work.[4] It is apparent then

[4] For example, in figure 3 at the time $t = 54$ minutes, where the flow is greatest and the kinetic energy should be at a maximum, the kinetic energy can be computed as follows:

that not only the size of the heart but the magnitude of the after-load, which is what the resistance to emptying really is, modifies the ability of the heart to utilize its energy for work.

Starling's law may thus be more precisely considered. While increasing heart size tends to increase heart work (1), and oxygen consumption (2), the increase in efficiency which is stated to occur (2) holds only when the after-load, the resistance to emptying, is not altered. Increasing the latter tends to reduce the mechanical efficiency and in our experiments this tendency was sufficient to nullify and sometimes even to overbalance the effect of increase in heart size tending toward increase in efficiency. It is obvious then that to define work, oxygen consumption and efficiency, it is necessary to consider the nature of the load as regards initial load (venous return), and after load (resistance to emptying), as well as in regard to heart size.

Clinically these facts are of significance since in disease, hypertension of the systemic and/or pulmonary circuits occurs with and without changes in cardiac output. Also, changes in cardiac output may occur with no change or an insignificant change in the after-load. The energy expenditure per unit of work would be different under these circumstances due to more than the change in heart size, assuming the same factors operate in man.

Our results permitted us also to demonstrate again the absence of a significant oxygen debt after the periods of increased work. Any oxygen debt is so small and fleeting as not to significantly alter the O_2 consumption after the heart had been restored to its preëxisting work level. This is in accord with the concept of Katz and Long (11) and confirms the observations on this point of Gollwitzer-Meier (3).

An interesting observation revealed by our experiments is the tendency of the oxygen consumption at the time of the 2nd sample to be less than at the time of the first sample during both periods where the work was increased. As a result there was an increase in efficiency as the increased work was maintained. This would imply that there is an adaptation to the increase in work on the part of the heart. In the instances of increase in initial load, the later O_2 consumption came to lie within the control values in some of the experiments. The nature of this adjustment is problematic.

It is possible that these factors of initial load and after-load operate also in the failing heart and failure to recognize this has perhaps been the cause,

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$K_E = \dfrac{Mv^2}{2g}$ gm. cm./sec., where M = mass of blood put out by heart per second; v = linear velocity of blood leaving heart in cm./sec.; g = 980 cm. sec.^{2}.

Where M = 2 times the pulmonary flow/sec. = 9.2 grams, and the diameter of the pulmonary artery and the aorta together is 0.6 cm. or more, K_E max. = 0.046/kgm. meters/hr. and $\dfrac{K_E \text{ max.}}{\text{work as calculated}} = \dfrac{0.046}{27} = \dfrac{1}{500}$ approximately.

among other things, of discrepancy in the results of studies on the failing heart. It is conceivable that in the failing heart even when these loads are unchanged the decrease in the ability of the heart to perform its work may make them relatively increased and hence under these circumstances they may come to represent the equivalent of an increase in load.

SUMMARY

A method is described for obtaining a true mixed coronary venous blood sample in the heart-lung or isolated heart preparation of the dog, and for calculating the true oxygen consumption of the heart.

Measurements of oxygen consumption and work of the heart preparation indicate that the increase in oxygen consumption consequent upon an increase in work depends not only upon the magnitude of the work increase, but also on whether the augmented work is produced by raising (1) the venous return or (2) the peripheral resistance. In the former case the increase in oxygen consumption is proportionally less than in the latter, so that a given amount of work is done more efficiently with a large venous return and a low peripheral resistance than the same amount of work done with a low venous return and a high peripheral resistance.

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THERMAL SENSATION AND DISCRIMINATION IN RELATION TO INTENSITY OF STIMULUS

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The object of this report is to present new data on the relationship of the intensity of a thermal stimulus to 1, the intensity of the sensory response, and 2, the discriminatory ability for temperature sensation.

The present work employs a modification of the radiation techniques formerly used in this laboratory (1) which lends itself especially well to this type of problem. The methods to be described use exclusively radiation as the stimulating agent, a procedure to which decided advantages accrue. It avoids any actual material contact between subject and stimulator, obviating thereby the arousal of unwanted sensations such as touch. This allows the subject to experience the desired thermal sensation undistracted by other factors. In addition, there are no mechanical effects resulting from this type of stimulus so that there is no interference with blood flow nor any distortion of the skin introduced as experimental artifact, whereas methods employing actual material contact of the subject with a stimulator cannot be wholly free from such effects. Besides having these advantages, the method is flexible and the stimuli and limens are easily made quantitative in the same absolute units. The Weber ratio thus obtained is therefore more significant than when it is obtained from measurements expressed in degrees Centigrade or other arbitrary systems.

Many studies of thermal discrimination and of the Weber-Fechner law have been made in the past, the net result of which has been to establish the Weber-Fechner law

$$S = k \log I + b \quad \ldots\ldots\ldots\ldots (1)$$

within certain limits, but to offer conflicting statements concerning the Weber law

$$\Delta I/I = C \quad \ldots\ldots\ldots\ldots (2)$$

In these equations, I is the intensity of the stimulus, ΔI is the least perceptible increment (that is, the least amount by which two stimuli can

differ in intensity and still be recognized as being different), S is the sensory response, while k, b and C are constants. The work of Culler (2) offers a possible clue to the reason for the confusion in data relating to the Weber law, for it leads to the conclusion that the Weber law is obeyed for absolute limens, i.e., for the smallest change in stimulus that can be perceived after the skin had been adapted to a given temperature level, but not at all for differential limens, that is, for differentiation between two stimuli acting on unadapted skin. The reference cited contains a bibliography up to 1926.

Hardy and Oppel (1) suggested that the Weber-Fechner law was obeyed for absolute limens (with the skin adapted to room temperature) within the comparatively narrow range of intensities in which they worked.

I. Sensory Response and Stimulus Intensity. Apparatus and procedure. Studies were first made with heat radiation which was periodically interrupted. The apparatus is shown in figure 1. Light from a 1,000 watt tungsten filament lamp, B, was focused by the lens, L, on the skin of the subject located directly behind the circular opening of the opaque screen, D. The size of this opening could be varied in discrete steps by the use of shields. A motor, M, through a suitable pulley arrangement, rotated the half-sector disc, S, so that each revolution of the sector gave an interval of radiation followed by an equal interval of dark. The speed of rotation could be controlled by the rheostats R and R^1. A rheostat (not shown in the figure) in series with the bulb, B, allowed the intensity of the light to be set at any desired value.

To insure that no radiation could penetrate beyond the surface of the skin, the forehead of the subject was blackened with India ink, and a central area was exposed to the radiation by placing the forehead just behind the opening of the screen, at F.

Intensity of radiation was measured by means of a radiometer which could be held at F when the forehead was removed. (In order to avoid cumbersome terminology, the radiation intensities will be reported as "units" where one "unit" is 10^{-5} gram calories per square centimeter per second.)

Previous workers (3) (4) have made use of intermittent radiation for the study of heat sensation. However, they did not measure the intensity of the radiation directly, but inferred ratios of intensity from current changes in the electrical circuit containing the heating element, and expressed their intensities in terms of the threshold intensity. Also, the importance of blackening the skin so as to localize the effect of the radiation at the surface and prevent penetration (5) does not seem to have been appreciated by these earlier experimenters.

When the forehead was in place, and the sector rotating, the exposed area gained heat during the interval of radiation and lost some of it (but not all)

during the time the radiation was cut off by the opaque half of the sector. Thus, for frequencies of rotation not too slow, the forehead would at first gain more heat than it lost, and its temperature would be fluctuating around a rising baseline. After a short time the forehead came to "equilibrium," it lost during the "dark" half of the cycle an amount of heat equal to that which it had gained during the "lighted" half so that its temperature

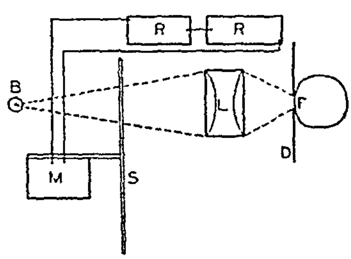

Fig. 1

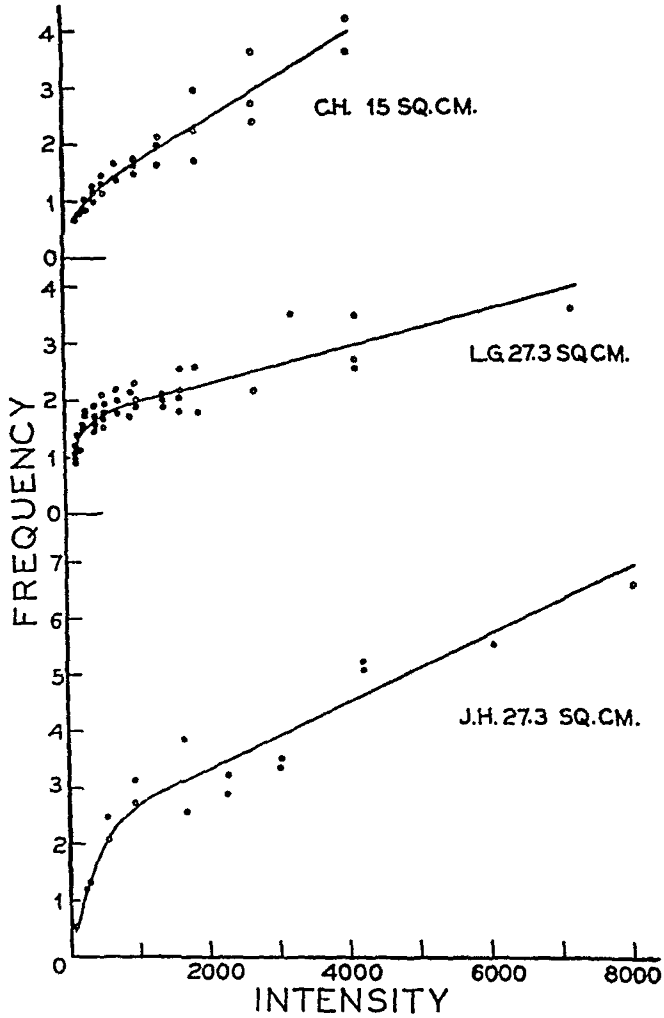

Fig. 2

Fig. 1. Diagram of the apparatus (meaning of letters explained in text).
Fig. 2. Fusion frequency (flashes per second) vs. units of intensity.

oscillated regularly along a steady baseline. It was after this steady state had set in that readings were taken.

The sensation accompanying this temperature oscillation was one of alternate pulses of warmth and cooling for the lower frequencies of rotation. As the frequency was increased, the warm pulses seemed to overlap, and the sensation became one of rippling warmth. By pushing the frequency still higher, this ripple was caused to become less and less distinct until

finally it fused into a sensation of continuous warmth. The frequency for which the ripple just disappeared was taken as the end point for the test. This end point is not a sharp one, and some practice is necessary if it is to be determined with a fair amount of certainty. For this reason the authors of this paper were the subjects and each one performed his own manipulation of the rheostat which controlled the rotation frequency. The end point for each of the tests in a run was taken a number of times and the results averaged: the extreme values of a set were rarely more than ± 6 per cent from this average. Suitable precautions were taken against returning by memory to a previous setting by changing pulley ratios, by blindly setting R^1 to an unknown value and adjusting the frequency using R alone, etc.

A. *Studies with flickering heat radiation.* Plots of the fusion frequency vs. intensity obtained in the manner described above are shown in figure 2.

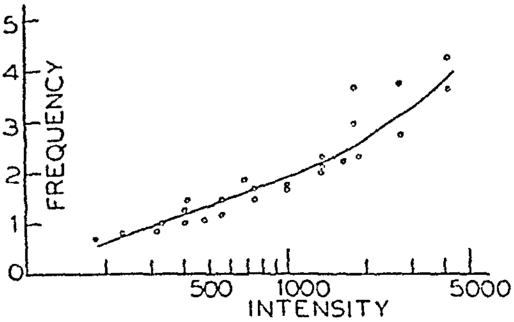

Fig. 3

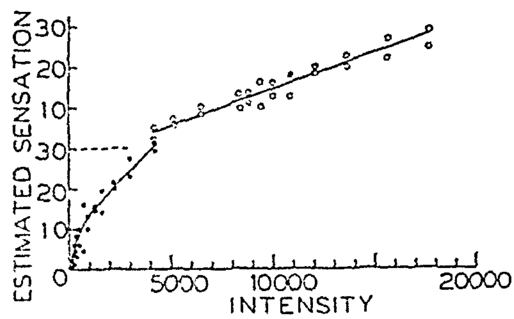

Fig. 4

Fig. 3. Fusion frequency (flashes per second) vs. units of intensity (logarithmic abscissa) for area of 15.0 sq. cm.

Fig. 4. Estimates of sensation vs. units of intensity. Data represent two separate runs (open and filled circles respectively).

The fusion frequency, after rising more or less rapidly at the lower intensities, swings into a gentler slope at roughly 1,000 units and is thereafter a linearly increasing function of intensity. The comparatively wide spread of the points is due in part to the fact that the curves are composites of runs taken on different days, and each observer was usually found to vary slightly in his level of sensitivity from day to day. One set of data (C. H.) is shown replotted against the logarithm of the intensity in figure 3. This shows a straight line up to about 1,000 units, followed by a non-linear portion. The significance of these plots will be discussed later, as they are intimately connected with the results presented in the next section.

B. *Studies employing graded estimations of sensation.* The relationship of the intensity of the sensory response to the intensity of stimulating radiation was tested in a manner similar to that recently used by Jenkins (6). A stimulus of 4,210 units was presented for two seconds to the subject and he was told to call the resulting sensation 10. He was

asked to bear this sensation in mind and to compare it with sensations which were to follow. An intensity which the subject felt to be half as strong as the standard he was to call 5, an intensity one-tenth as strong, 1, etc. He was then stimulated with intensities ranging from 83 to 4,210 units in a random manner, being always in ignorance of the intensity selected and of his progress in the test. The cycle of intensities was repeated three times, each time in a different order. Between the tests the subject was allowed to rest, and at the beginning of each set the standard intensity was given to refresh his memory.

The individual's score was added for each intensity. The results of this test are shown as the first part of the curve of figure 4 (filled circles), each circle representing the sum of the three separate estimations of sensation of one individual plotted against the intensity of the corresponding stimulus. A similar test was made separately in the range 4,260 to 17,640

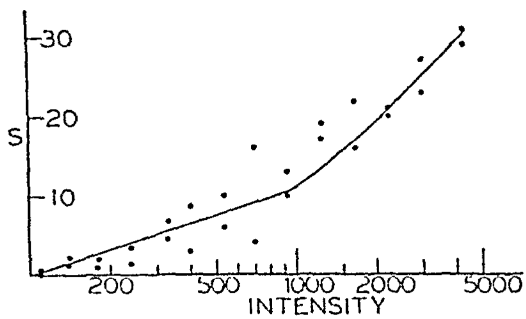

Fig. 5

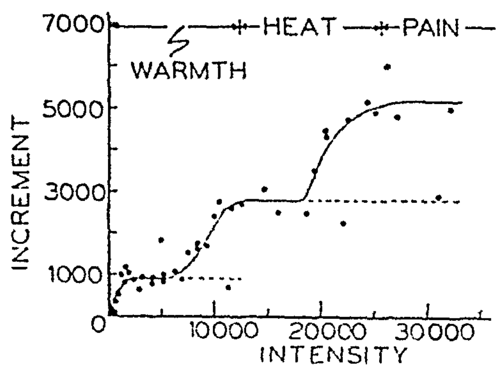

Fig. 6

Fig. 5. Estimates of sensation, S (ordinate) vs. intensity (logarithmic abscissa).
Fig. 6. Least perceptible increment (Weber increment, ΔI) in "units" vs. intensity in "units."

units, the latter being assigned the value of 10. The sensation rose linearly with the intensity as may be seen from the second part of figure 4 (open circles). This second part is obviously an extension of the first.

The similarity of the curves in figure 2 to that in figure 4 is at once apparent. Both show an initial rise up to 1,000 units followed by a linear portion whose slope is less than that for the portion of the curve below 1,000 units.

When treated in a slightly different way, the data for the preceding test will show clearly several points. Figure 5 shows the estimates of sensation as linear ordinates plotted against stimulus intensity on a logarithmic abscissa. Here again this test gives a curve remarkably similar to the corresponding treatment of the flicker data (fig. 3). Both show straight line portions up to about 1,000 units, followed by a non-linear curve. It will be seen on closer inspection that the two curves are practically superimposable point for point, if the arbitrary scale for sensation be adjusted

by multiplication by a scale-constant. The conclusion can then be drawn that the fusion frequency is truly representative of the sensory response.

The straight line plot of sensory response vs. intensity on a logarithmic scale below 1,000 units, as shown in figure 5, indicates adherence to the Weber-Fechner law. Likewise the fusion frequency data of figure 3 can be represented below 1,000 units by the Ferry-Porter law,

$$N = a \log I + d$$

where N is the fusion frequency, I the intensity, and a and d are constants. Since figures 3 and 5 show such close similarity, it is clear that the Ferry-Porter law is a variation of the Weber-Fechner law. When the results of the next section are presented it will be seen that the change in slope of these curves around 1,000 units is of physiologic significance.

II. *Thermal Discrimination.* The flicker method does not allow of direct measurement of the Weber increment (least perceptible increment), so for a direct evaluation of ΔI, recourse was had to the following method:

The rotating sector was set at a constant frequency of 0.5 cycle per second, and again a shutter was interposed so that by lifting it the operator could present a single stimulation pulse lasting two seconds. For low intensities the 15 cm.^{2} opening was used. A thin metal rod, to which was fastened a semicircular disc of copper, was mounted before the opening in such a manner that it cut off radiation from half the area, and could be flipped from side to side so that either the right half or left half of the area was darkened. A measured stimulus, I, was then presented to the subject on one side, and after a short pause a slightly greater stimulus, I_2, was presented on the other side. This test was repeated with the second stimulus being made larger than the first by small steps until the subject indicated that he could just distinguish between the two. In this way the least perceptible increment was directly obtained:

$$\Delta I = I_2 - I_1$$

For high intensities this method was modified in part to avoid stimulation of too large an area with a strong stimulus (sometimes the sensation was painful). Also, at high intensities an asymmetry in responding end organs became noticeable over the central portion of the forehead although no reliable indications of such an asymmetry could be obtained for this area at low intensities. The modification was this: The flip disc was removed, an area of 3.5 cm.^{2} used, and the two stimuli were presented over the same area with an interval of 30 to 60 seconds between them.

To insure that these two variations of the method were consistent with each other, they were used in an overlapping region (5,000 to 6,400) and were found to give identical results. The skin on which these tests were made had been adapted to room temperature, and it should be remarked

that the short duration of stimulus flash in the procedure allowed little adaptation to the radiation to occur.

Thus, of the results reported, those up to 5,000 units were obtained by the first method, those from 5,000 to 6,400 units by either of the two, and those past 6,400 units by the second method. Figure 6 shows a plot of ΔI vs. I. The curve is readily broken up into six regions. In region A, ΔI is a linear function of I, increasing as the intensity of the stimulus is increased. In region B, ΔI remains constant at a value of about 890 units. In region C, ΔI again assumes increasing values as the stimulus is increased, becoming constant in region D at a value of 2,670 units. In region E, ΔI once more resumes its upward trend with increasing intensity of stimulus and finally levels off in region F to a constant value of about 5,050 units.

To assure that there was no asymmetry introduced into the results by the method, tests were made to determine whether the decrement from a high to a lower intensity was the same as the increment from a low to a higher. There was found no difference between increment and decrement, i.e., the same number of units of intensity had to separate two stimuli if they were to be distinguishably different, no matter whether the higher were gradually decreased or the lower increased, a result which, of course, was expected.

The behavior of the Weber increment shows that the discriminatory ability of the skin is best when the discrimination is to be made at temperature levels close to the normal skin temperature, that is, for low intensities. As the intensity of the stimulus increases, the skin loses relatively more of its discriminatory powers. This loss does not proceed indefinitely, however, since the Weber increment becomes constant at stimuli between 1000 and 1500 units and presumably would remain so were the situation not complicated by the entry of other factors. For the particular circumstances of this experiment, the discrimination at the skin temperature levels is such as to allow a change in skin temperature of 0.002°C. per second to be detected after two seconds. The level of constant discrimination is attained for temperatures approximately 0.2°C. removed from skin temperature; here a change in skin temperature of 0.06°C. per second can be detected after two seconds. This degree of discrimination holds for temperatures as far removed as ±1.0°C. from skin temperature. It must be remembered that these propositions are valid only for a purely thermal stimulus such as heat radiation. Other circumstances would prevail with thermal stimuli accompanied by touch.

In regions A, B and C the sensation was one of warmth—a mild, pleasant, diffuse sensation. Just at the start of D the sensation assumed the quality of heat; this was sharper and sometimes stinging, but still diffuse and it continued through D and E. At the start of the F region the sensation became a painful one: sharp, biting, and granular. The heat and pain

thresholds were determined experimentally by presenting to the subject a series of intensities scattered randomly and asking for a report as to the quality of the sensation. The intensities at which the sensations of heat and pain first made their appearance were quite definite.

Conjecture as to the causes for the general shape of the curve may be made along the following lines. In region *A* there may well be operative some kind of statistical law governing the responses of the end organs—such a law might easily result from the nature of the matrix surrounding the end organs. It seems very likely that a group of receptors such as those in the skin, surrounded by a system of capillary pipes through which blood is coursing, near sweat glands which doubtless have intermittent periods of activity, and imbedded at different levels, should by the very nature of their position and of the inhomogeneity of their surroundings differ somewhat in their excitability; differ, that is, from each other and also show individual variation as time goes on. An interpretation along these lines is that in region *A* the warmth end organs which effectively respond to the stimulus increase in number as the intensity is raised until at its end the whole number is responding at least minimally.

That something of this nature is the case may be gathered from the work of Geblewicz (7) who measured the time required for a thermal stimulus to arouse maximum sensation and found that this time was longer, the greater the stimulus intensity. Such a result would indicate that the higher intensity is gradually bringing into activity those end organs which, probably because they lie deeper, are not so quickly excited by the stimulus and those which, because of other variable factors, have a higher threshold. Further, the change in slope of the curves obtained by the flicker method and by estimates of sensory response supports the idea that around 1,000 units or so a definite change in response occurs. In region *B* the skin is evidently detecting an absolute difference and not a fractional increase. This may be due to the fact that now all end organs are responding superminimally, although the reason why this should give a constant increment is obscure if, indeed, the mechanism proposed obtains. Region *C* denotes the entrance of a new factor (*C* factor) as is evidenced by resumption of rise of the increment. However, in this region the conscious sensation is still that of warmth and it is not until *D* is reached that sensation of heat actually is identified as such.

Possibly *C* and *D*, as well as *E* and *F*, are mechanisms analogous to *A* and *B*. In *E* and *F*, although the *E* factor enters to cause the increment to rise progressively above the flat *D* region, the sensation of pain does not become evident until *F* is reached.

Occasionally a point is obtained which lies comparatively far off the best curve which can be fitted to the data. When this deviation is towards a higher increment than is normal for that region, the explanation most

likely is that some sweating occurred, which would thus raise the increment above its true value. This explanation cannot hold, however, for some of the points which lie well below the curve. The obvious explanation for these latter is that the area stimulated, by chance, contained no active end organs of a given type; therefore, the system was thrown back to the next lowest level. To take a definite example, consider the point at 31,250 units. If during this test there were no pain receptor available for excitation, then the sensation would be one of intense heat corresponding to excitation of the warmth receptors and the *C* factor; and, indeed, for this case the point lies tolerably well on the dotted extension of the flat region proper to them. The fact that a few times a "renegade" sensation was obtained (heat, for instance, where there should have been pain) lends credence to such belief.

It might be inferred from this that the *AB*, *CD* and *EF* regions are really in a sense distinct curves superimposed one on the other, and that the break from the flat portion of one to the rising portion of the next is really a sign of the entry of a new factor into the sensory field. It is worthy to note that precisely at the bends did the subjects show most uncertainty during the tests and that the spread was greater here than elsewhere.

The trend of the curve makes evident an impairment of the discriminatory sense just before the new sensation is recognized. This is an important consideration, as it indicates that the threshold of *excitation* for both heat and pain probably lies below the actual *sensory* threshold for these sensations, an expected result. A study of this kind may objectively reveal end organ activity which will eventually be forced on the consciousness as a sensation when its intensity becomes high enough. Thus, before the subject is aware of either heat or pain sensation, sub-sensory responses of these modalities might be detected.

Although Hardy, Wolff and Goodell (8) report the pain threshold as 24,000 units, it is probable that the pain neurones are excited by 18,500 units. Similarly, the heat threshold is probably about 5000 units lower than that at which the sensation is first perceived. Further, the conclusion that the excitation threshold for pain is lower than the sensory threshold lends added support to the contention of the above authors that pain shows no spatial summation. These workers found that the pain threshold did not decrease when larger areas were stimulated but were not able to prove that the sensory threshold was not also the excitation threshold for pain. The present work indicates that the pain receptors are already aroused to sub-sensory activity before the sensory threshold is reached. Therefore, increasing the area would demand reduction of the stimulus intensity necessary to elicit threshold pain, had pain the property of spatial summation.

Figure 4 gives the subjective estimate of sensory response to the intensity

of stimulus presented, and covers the region from threshold well into the *D* portion of figure 6. Sensation seems to increase without regard to the behaviour of the Weber increment. However, it is not surprising that the *C* region does not show on figure 4, for here the increment is scarcely smaller than the extent of the region itself. The situation could not be remedied by taking a large number of points close together, for the steps would be separated by less than the necessary increment.

It appears certain that the intensity of the sensory response increases constantly as the stimulus is made progressively stronger, even in the regions where the Weber increment is constant.

That there is no conflict between figures 4 and 6 becomes apparent when it is realized that figure 6 is really the slope of the sensation curves of figure

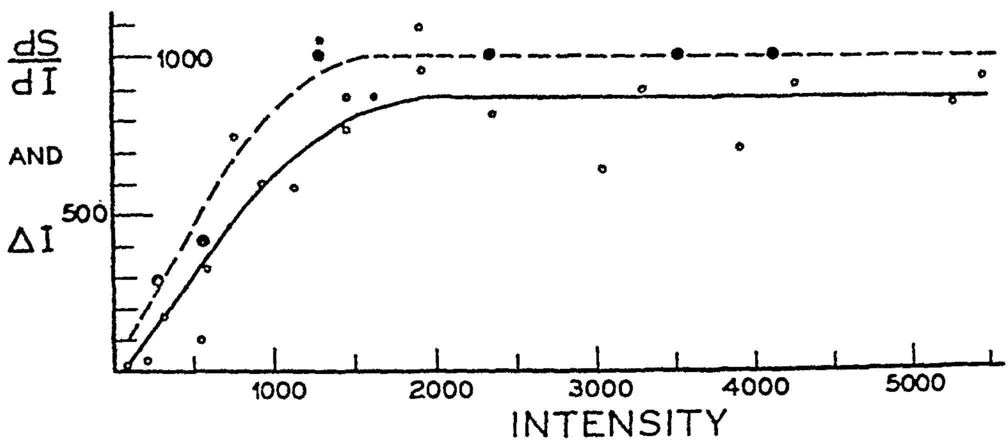

Fig. 7. dS/dI is the slope of the sensation vs. intensity curve (fig. 4) (solid circles); ΔI is the Weber increment (open circles). The former quantities were multiplied by an arbitrary constant to adjust them to fit the scale for ΔI. Both quantities are then plotted as ordinates against units of intensity as abscissae.

4. If the slope of figure 4 be taken at convenient points and plotted with the corresponding range of figure 6 as was done to obtain figure 7, the points are seen to be entirely consistent with the curve for the Weber increment. In constructing figure 7 the slopes from figure 4 were multiplied by an arbitrary constant to adjust the scale for comparison. This can be done without affecting the validity of the comparison as the scale for the sensory evaluation was purely arbitrary.

It is an interesting point to note that figure 6 allows one to determine how many discriminable steps of heat can be distinguished from threshold stimulus to a painful stimulus. If the threshold be taken as the lowest possible, i.e., the excitation threshold of the receptors, then there appear to be about 30 steps of thermal sensation distinguishable between this threshold and the onset of pain.

SUMMARY

1. The flicker method for studying temperature sensation is presented and is found to give results in harmony with results obtained by direct estimates of sensation. Wider use is made of the subjective estimates of degrees of sensation than has hitherto been generally attempted, and this method has been found thoroughly reliable.

2. The Weber-Fechner law was found to hold in a limited range (up to $1{,}000 \times 10^{-5}$ gm. cal. per sq. cm. per sec.).

3. The Ferry-Porter law is demonstrated to be a special case of the wider Weber-Fechner law.

4. A study of the Weber increment shows that it may either change with the intensity, or be a constant, depending on the range. The behavior of the increment is such as to suggest three receptor types: warmth receptors, pain receptors, and a "C receptor."

5. A close correlation between Weber increment and sensation increment has been made for warmth.

6. There are about 30 discriminable steps in the range of intensities from threshold to pain.

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THE PANCREATIC SECRETAGOGUE ACTION OF PRODUCTS OF PROTEIN DIGESTION

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Whether products of protein digestion contribute directly to the stimuli causing the intestinal phase of pancreatic secretion has not been determined. They are known to increase pancreatic secretion when taken by mouth (Dolinski, 1894; Bylina, 1911; Kobzarenko, 1915) but their effectiveness is generally attributed to the acid which they cause to be secreted by the stomach or to the water contained in their solutions (Babkin, 1914; Ivy, 1930). In a few experiments amino acids (Frouin, 1913; Arai, 1921) or peptones (Conheim and Clee, 1912) have been introduced directly into the intestine and stimulation of pancreatic secretion observed. The solutions used in some of these experiments were strongly acid. Neutral solutions were reported to have about the same effect as an equal amount of water. No technically satisfactory experiments have been reported. Many were done on anesthetized animals, generally the pH and freezing point of the solutions were unknown and in some the methods of collecting pancreatic juice were not such as to give reliable quantitative results. For this reason and in view of the recent observation (Crider and Thomas, 1940) that the water present in neutral, isotonic solutions fails to stimulate the pancreas when placed directly in the intestine, we determined to reinvestigate the problem.

Methods. The arrangements for collecting pancreatic juice were the same as described in previous reports (Crider and Thomas, 1940; Thomas and Crider, 1940; Thomas, 1941). Four dogs were used but the quantitative data to be presented were obtained on three only, one having died before the work was completed. Each animal was provided with a cannulated duodenal fistula placed opposite the opening of the main pancreatic duct and the juice was collected through a rubber funnel held against the mucosa surrounding the duct. Appropriate control experiments proved that extraneous fluid (succus entericus?) collected by this method amounted to less than 1 cc. per hour and was not increased by injection of peptone or amino acids into the intestine. A cannulated gastric fistula was also made and effective drainage of the stomach was maintained during experimental periods. In one dog the bile duct was

transplanted into the stomach and in this and one other animal the accessory pancreatic duct was ligated at the time of operation. Much larger quantities of pancreatic juice were regularly obtained from the animals with accessory ducts ligated. Qualitatively, the results were uniform regardless of the type of operation.

Experiments were begun 18 to 24 hours after the last previous meal. Solutions were introduced into the intestine via a small ($\frac{1}{16}$ inch bore) rubber tube passed distally through the duodenal fistula to a point 6 inches to 12 inches below the pylorus.

The effect of peptone in the intestine was also tested on a few anesthetized animals but with uniformly negative results.

RESULTS. *The possible rôle of bile in the secretagogue action of peptone.* Very early in this study it became evident that introduction into the intestine of neutral, isotonic solutions of commercial peptones, certain amino acids, or protein digests prepared in the laboratory was followed regularly by an increase in the rate of pancreatic secretion. However, peptone in the intestine also stimulates the flow of bile (Bruno, 1899; Pavlov, 1910) and bile was thought by some to stimulate the flow of pancreatic juice (Mellanby, 1926). We therefore thought it advisable to postpone further study until the question of the pancreatic secretagogue action of bile could be investigated. Experiments of which a preliminary report has been made (Thomas and Crider, 1941) proved conclusively that, under our experimental conditions, bile in the intestine does not increase the flow of pancreatic juice.

Comparison of peptone with other stimuli. In this series of experiments the effect of injecting 20 cc. of a 5 per cent peptone (Bacto-Protone, Difco.) solution into the duodenum was compared with the effect of 10 cc. of N/10 HCl, or 40 to 60 cc. of distilled water, similarly administered, and with 4 "units" of a commercial secretin preparation (Pancreotest) given intravenously. The volume, specific gravity and total nitrogen of the pancreatic juice collected after stimulation were determined.

A consistent routine was followed in all the experiments. After the animal was prepared a single injection of one of the stimulating solutions was administered and after a convenient time, never more than one minute, a 10 ml. graduated cylinder was placed under the collecting tube and the secretion collected for 10 minutes. Again after a convenient time, generally one minute, a second injection of the same material was given and the above described procedure repeated until five or six samples had been obtained. Another stimulus was then substituted and the procedure repeated until the desired number of samples had been obtained with each stimulus. In a few experiments the secretion was collected for several minutes after feeding and studied to determine the properties of "psychic secretion."

The first few observations revealed the fact that although the properties of the secretion varied with the type of stimulus used, characteristic values were not obtained until at least two injections of the same stimulus had been given. Consequently, all samples obtained during the 10 minutes following the first injection of any stimulus were discarded and are not included in the reported data. Volume and specific gravity were determined on each sample and all but the first sample obtained during the use of a single type of stimulus were pooled for nitrogen determination. The order in which the various stimuli were used was varied from day to day.

The nitrogen determinations were made by a trained technician using the micro-Kjeldahl method. The method as used appeared to be subject

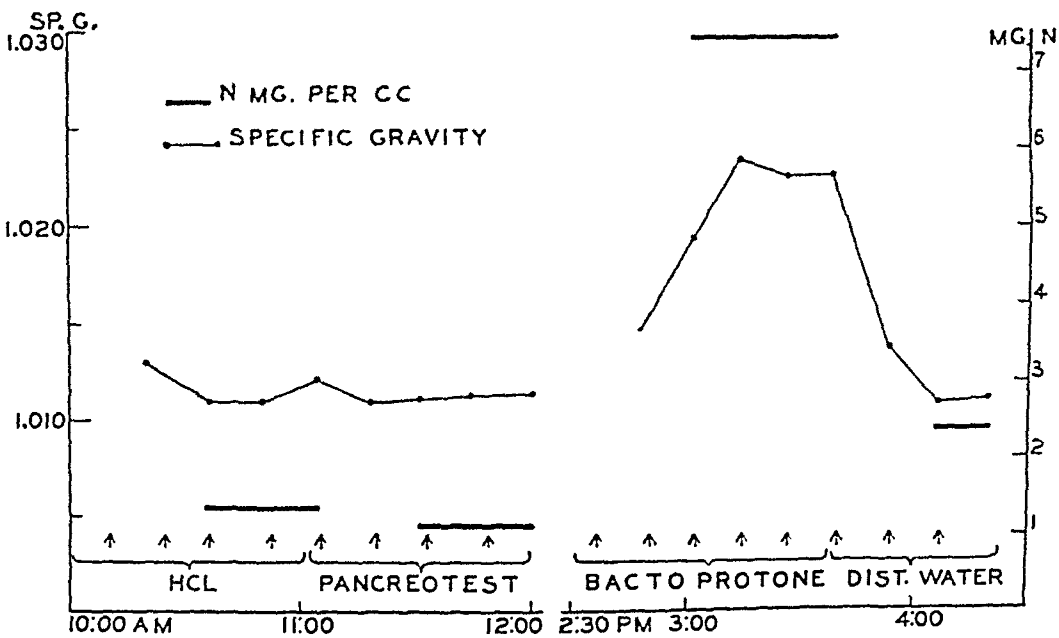

Fig. 1. Graphic protocol of a typical experiment on dog 2-40 showing specific gravity and total nitrogen (mgm. per cc.) of the pancreatic secretion obtained with various stimuli. The small arrows mark the moments at which injections were given.

to a maximum error of about 15 per cent. Specific gravities were measured by means of a 1 ml. specific gravity bottle.

The procedure and results of a typical experiment are illustrated graphically in figure 1. Average data obtained in all the experiments of this series are presented in table 1. It is evident that 20 cc. of peptone solution caused the production of a slightly larger amount of secretion than 40 to 60 cc. of distilled water. Also the secretion following peptone administration had a higher specific gravity and contained several times as much nitrogen per cubic centimeter as that caused by water, secretin, or HCl.

Comparison of various protein digests. This series of experiments was undertaken primarily to determine whether the capacity to stimulate the

secretion of pancreatic juice was limited to a few peptones or was characteristic of protein digests generally. As the work progressed it became evident that some digests were more effective stimuli than others so the data were arranged to bring out that fact as well. The experiments were conducted in the same manner as those in the group just preceding. Except in a few preliminary experiments, the total experimental time in any

TABLE 1

Amount and properties of pancreatic secretion produced by various stimuli

STIMULUS	DOG NUMBER	VOL. PER 10 MIN. SAMPLE	SP. G.	TOTAL N	TOTAL N	NUMBER OF SAMPLES
				mgm./cc.	*mgm./sample*	
10 cc. N/10 HCl into duodenum	3-39	7.47	1.0119	1.54	11.5	4
	2-40	8.6	1.0103	1.38	11.8	6
	3-41	10.0	1.0099	0.84	8.4	6
Average		8.69	1.0107	1.28	10.56	
Secretin (4 units of pancreotest) intravenously	3-39	4.3	1.0133	—		4
	2-40	9.13	1.0113	1.12	10.22	3
	3-41	7.6	1.0111	0.42	4.19	4
Average		7.01	1.0118	0.77	7.20	
Dist. H_2O 40 to 60 cc. into duodenum	3-39	0.96	1.0112	—		2
	2-40	4.4	1.0110	2.38	10.38	2
	3-41	3.03	1.0127	2.66	8.06	6
Average		2.79	1.0116	2.52	9.22	
20 cc. 5 per cent bacto protone into duodenum	3-39	1.54	1.0365	16.96	26.12	5
	2-40	3.73	1.0224	6.94	26.6	13
	3-41	3.9	1.0174	5.9	21.09	13
Average		3.06	1.0254	9.930	24.61	
Feeding (psychic secretion)	3-39	—	1.0216	—	—	1
	2-40	—	1.0190	4.44	—	5
	3-41	—	1.0120	—	—	1
Average		—	1.0175	4.44	—	

one day was limited in this series to about three hours because it was found that the secretion obtained after prolonged stimulation had a lower specific gravity and contained less nitrogen than that obtained earlier.

The various substances used are listed in table 2. The commercial peptones were prepared in 5 per cent solution in 0.6 per cent sodium chloride solution. Freezing points were not determined but we know from pre-

vious experience that such solutions are approximately isotonic. The pH was determined for a sample of such a solution of each peptone; all were above pH 6.0. The digests prepared in the laboratory contained 4.0 mgm. of nitrogen per cc. and approximately 2½ per cent of solids. They were adjusted to between pH 6.8 and 7.2 with NaOH or HCl and made isotonic

TABLE 2

Amount and properties of pancreatic secretion produced by various protein digests

STIMULUS			SECRETION					RATIO, N SECRETED: N INJECTED
	Total N	Per cent proteose	Number of samples	Vol. per 10 min. sample	Sp. g.	Total N	Total N	
	mgm./cc.			*cc.*		*mgm./cc.*	*mgm./sample*	
Commercial products								
Witte's peptone.	5.95	(high)	13	2.93	1.0249	9.60	24.01	0.201
Bacto protone..	7.92	81+	31	3.06	1.0254	9.93	24.61	0.155
Neo peptone....	7.42	25+	14	2.35	1.0209	8.20	17.71	0.133
Bacto peptone..	8.26	6−	42	1.83	1.0226	8.86	14.47	0.092
Amino acid powder (Stearns).....	6.46	0	11	1.08	1.0258	4.88*	6.29*	0.048*
Laboratory preparations								
Pepsin digest of casein........	4.0	—	21	2.27	1.0218	7.526	15.541	0.194
Pepsin-trypsin digest of casein........	4.0	—	22	1.96	1.0244	8.71	14.30	0.178
Proteose from neo peptone...	4.0	100	24	2.39	1.0205	6.48	13.88	0.173
Proteose from intestinal contents.........	4.0	100	12	2.30	1.0202	6.39	13.27	0.164
Proteose from gastric contents.........	4.0	100	11	1.96	1.0199	7.00	12.55	0.156
P.T.A. digest of casein........	4.0	(trace)	10	1.99	1.0233	5.5*	12.07*	0.15*

* Average based on data from 2 animals only.

(Δ between 0.5° and 0.65°C.) by addition of NaCl when necessary. The pepsin and pepsin-trypsin digests of casein were prepared as described in a previous article (Thomas and Crider, 1939). The "P.T.A." (pepsin-trypsin-alkali) digest was prepared by subjecting the pepsin-trypsin digest to prolonged boiling after saturating the solution with barium hydroxide.

The amino acid powder[1] gave a negative biuret test but was not guaranteed to be free of peptids. All solutions were injected in 20 cc. amounts.

The results are summarized in table 2. The figures presented are averages of the results obtained on the three dogs. There were consistent differences in the responses of the individual dogs, an estimate of which can be obtained from the data in table 1. The results recorded in the table show that all the preparations tried in this group of experiments were effective in causing secretion of a considerable amount (averaging 1 to 3 cc. in 10 min.) of pancreatic juice of high specific gravity and containing a large amount of nitrogenous material.

When preparations of uniform concentration in terms of nitrogen, such as the laboratory preparations, were used the stimulating activity of the various products may be compared by comparing the volume of the 10 minute samples or the total nitrogen secreted (mgm. per sample). For comparing these preparations with the commercial products, which were used in more concentrated solutions, the figures given in the last column of the table are presented. These values were obtained by dividing the figures for total nitrogen secreted (mgm. per sample) by those for total nitrogen injected (mgm. per 20 cc. of solution). Obviously, this method of comparison fails to take account of any influence which concentration alone independent of total quantity may have had on the effectiveness of the material.

It may be noted that differences in the effectiveness of the various peptone preparations as stimuli for the pancreas appeared chiefly as differences in the volume of secretion; specific gravity and total nitrogen (mgm. per cc.) showed little variation in the averages. We also noted that among the individual animals those yielding a larger volume of secretion in response to a given peptone stimulus produced proportionately less nitrogen in milligrams per cubic centimeter so that the total nitrogen secreted (mgm. per sample) in response to a uniform stimulus was remarkably similar from animal to animal. This fact is well brought out in the results with Bacto-Protone, presented in table 1.

Discussion. These experiments prove that various products of protein digestion are capable of acting in the intestine as powerful stimuli for certain functions of the pancreas, notably the secretion of nitrogenous solids. These products also increase the volume of the pancreatic secretion but in this respect they are inferior to HCl. It has also been shown that the secretagogue action of peptone solutions is not dependent on the water present nor on acid which they cause to be secreted. It is, therefore, a specific property of one or more of the products formed in the digestion of protein. What the active products are is difficult to determine with

[1] This material was kindly furnished gratis by Frederick Stearns and Co. of Detroit.

certainty but purified proteoses and some of the amino acids have been shown to be effective stimuli. On the other hand, the most abundant amino acids (glutamic acid and glycine) are known to be ineffective in neutral solution (Thomas and Crider, 1940) and some of the peptones and peptids may, therefore, also be inactive. However, the fact that eleven different preparations derived from various proteins and subjected to various degrees of digestion by different agents all exhibited the capacity to stimulate the pancreas indicates that this property is widespread among the products of protein digestion.

Among the commercial preparations the capacity to stimulate the pancreas appears to be inversely related to the relative completeness of digestion as indicated by the diminishing percentage of proteose. This fact suggests the attractive theory that only those products which require further digestion call forth an abundant secretion from the pancreas. Results obtained with the laboratory preparations do not seem to support this suggestion. For example, the proteoses were no more effective stimuli than the pepsin-trypsin digest of casein and of the three casein digests only the pepsin-trypsin-alkali (P.T.A.) digest appears, on the basis of data obtained from two animals, to have lost potency as a stimulus with the progress of digestion. There is reason to believe that data from the other animal would have diminished this apparent loss. The relative effectiveness as stimuli for the pancreas of the various classes of products of protein digestion must, therefore, remain undecided for the present.

The mechanism through which the peptones stimulate the pancreas has not been studied by the usual methods. Nevertheless, the properties of the secretion are such that it could not have been produced by the action of secretin alone. The only alternative mechanism known is nervous and it is significant that the properties of the secretion obtained by peptone stimulation are similar to those of "pilocarpine juice," "vagus juice," "psychic secretion" (see table 1) and the secretion following administration of acetylbetamethylcholine (mecholyl). We are, therefore, convinced that the peptones act through a nervous mechanism. If we are right we have, we believe for the first time, found a function of major physiological importance to be ascribed to the secretory nerve fibers that have long been known to supply the pancreas. We hope in the near future to begin experiments designed to trace the reflex pathway.

SUMMARY AND CONCLUSIONS

1. Products of protein digestion act in the intestine as stimuli for the external secretory function of the pancreas. Their effectiveness is not dependent on the coincident flow of bile into the intestine nor on the secretagogue action of water or acid.

2. The secretion produced by peptone stimulation has a higher specific

gravity and contains many times more nitrogen per cubic centimeter than that produced by water, acid or secretin.

3. There are wide variations in effectiveness as stimuli for the pancreas among various commercial peptones and protein digests prepared in the laboratory. Among commercial peptones the more effective products are those having the higher percentage of proteose.

4. The properties of the secretion are such that it could not be produced by secretin stimulation alone.

5. The secretion elicited by peptones resembles that caused by pilocarpine and other stimuli acting through or on the secretory nerves. Hence, the conclusion is drawn that the peptones act through a nervous mechanism.

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INFLUENCE OF PHYSICAL WORK ON PHYSIOLOGICAL REACTIONS TO THE THERMAL ENVIRONMENT

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Object of study. Previous studies from this laboratory have analyzed in some detail the reactions to various atmospheric conditions of the human body when at rest in a semi-reclining position (Winslow, Herrington and Gagge, 1937; Herrington, Winslow and Gagge, 1937; Gagge, Herrington and Winslow, 1937; Winslow, Herrington and Gagge, 1939; Gagge, Winslow and Herrington, 1938; Winslow, Herrington and Gagge, 1938; Winslow, Gagge and Herrington, 1939; Winslow, Gagge and Herrington, 1940). The purpose of the present investigation was to extend the observations made on resting subjects to subjects performing active physical work on a bicycle ergometer.

Conduct of experiment. The studies were carried out in an experimental booth which has already been described (Winslow, Gagge and Herrington, 1940) and by the same general techniques employed in our earlier work.

Air and wall temperatures were the chief atmospheric variables studied in the investigations here reported. They were accurately regulated and checked by records of air temperature and measurement of the brine temperature in the walls of the chamber (which determined mean radiant temperature) at twenty-minute intervals. The relation between brine temperature and mean radiant temperature was, of course, previously established by Vernon (1932) globe readings. Atmospheric humidity was maintained in general in the tests between 40 and 50 per cent saturation. Air movement in the chamber was measured by the hot-wire anemometer and was ordinarily 5 to 8 cm. per sec. In certain experiments, as noted, higher velocities were produced by the use of fans.

Two subjects (nos. VII and IX) were used in all tests, the same young men employed in earlier studies (Winslow, Gagge and Herrington, 1940).

The unclothed subject, after preliminary weighing, took his place on a bicycle ergometer of the recording electrodynamic type described by Kelso and Hellebrandt (1934), and began exercising at a predetermined load, measured on a recording voltmeter, and a fixed pedalling rate. Each experiment lasted for 90 minutes and computations of heat interchange were based on the period between $22\frac{1}{2}$ and $82\frac{1}{2}$ minutes from the beginning of the experiment.

The schedule of an experiment was as follows:

a. Rectal temperatures were recorded by a thermocouple inserted into the rectum at the beginning of the experiment and the measurement repeated every $7\frac{1}{2}$ minutes thereafter.

b. Skin temperature at fifteen representative points on the body surface was recorded at half-hour intervals by a Hardy thermopile.

c. Total metabolism (O_2 consumption) was determined twice with 15-minute samples, between $22\frac{1}{2}$ and $37\frac{1}{2}$ minutes and between $67\frac{1}{2}$ and $82\frac{1}{2}$ minutes after the beginning of the experiment, by means of a special adaptation of the Benedict-Roth apparatus, capable of handling rates up to ten times basal values (Gagge, 1941).

No determination of R.Q. was made since it appeared that the possible error in computing the caloric equivalent of the O_2 consumption on the basis of an assumed R.Q. of 0.83 would be very small. The typical exercise R.Q. has been shown to be affected by special diets and has been the subject of considerable dispute. Two recent studies (Christensen and Hansen, 1939a, 1939b) on subjects trained for ergometer work and living on a mixed diet have shown, however, that 0.83 is a representative value. Other data reported by the same investigators and also by Dill, Edwards, Bauer and Levenson (1931) have likewise shown that R.Q. is not affected by variations in environmental temperature over the range from 7 to 34°C. The inherent convenience of a record based on oxygen consumption alone was thought to outweigh, for our particular experimental aims, any minor increase in accuracy possible with gas analysis techniques.

d. The subject was weighed at the beginning of each experiment, after 15 minutes, after 45 minutes, and finally at the close of the experiment, on a sensitive platform scale placed adjacent to the bicycle.

Determination of convection constant. Of the five fundamental elements in thermal interchange, metabolic heat production and evaporative heat loss were thus obtained by direct observation. Convection and radiation interchanges are, of course, determined by air and wall temperatures, respectively, on the one hand and mean skin temperature on the other, which were recorded as indicated above. To determine the factor of heat loss by convection per degree difference between mean skin temperature and air temperature, the following series of experiments were conducted.

Wall temperatures were so regulated as to be approximately the same as mean skin temperatures, so that radiation interchange was practically nil. Air temperature, on the other hand, was varied over a range of 15°C. Observations were so made that the partitions applied to approximate "steady states"; hence thermal storage was usually insignificant. Under such circumstances, storage can be computed with reasonable accuracy from the changes which occur during the experiment in skin temperature and rectal temperature, giving the former a weight of 1 and the latter a weight of 2.

In the six experiments with each of two subjects, presented in table 1, it will be noted that work was limited to approximately 46.3 ± 0.2 kgm.-cal. per hour and the corresponding metabolism ranged between 297.0 and 328.2 kgm.-cal. Mean radiant temperature and mean skin temperature never differed by more than 1.6° and radiation heat interchanges, therefore, varied only from + 7.6 to − 13.4 kgm.-cal. Storage exceeded 9 kgm.-cal. in only two instances and in only one of these exceeded 13 kgm.-cal. This was because the skin temperature of the subjects never changed more than about 1° and the rectal temperature never more than 0.5°. Thus, the con-

TABLE 1

Experiments conducted for the determination of the convection constant

SUBJECT AND EXPERIMENT	TEMPERATURE, °C.			HEAT INTERCHANGE, KILOGRAM-CALORIES PER HOUR					
	T_A*	T_W*	T_S*	M†	W‡	E§	R¶	S‖	C**
VII-77	15.7	30.2	31.7	297	−46	−73	−10	−6	−161
78	16.7	32.1	32.3	315	−46	−127	−2	−9	−132
79	19.6	30.8	32.2	313	−46	−114	−11	−3	−139
80	23.3	31.2	32.8	304	−46	−153	−12	−10	−82
81	28.3	32.3	33.9	316	−46	−193	−12	−10	−54
82	32.1	34.3	34.3	316	−46	−234	0	−9	−26
IX-63	17.1	32.4	31.3	307	−46	−118	7	3	−153
64	17.9	31.4	31.8	322	−46	−106	−3	4	−171
65	21.3	32.7	31.6	300	−46	−135	7	3	−124
66	24.1	31.8	32.0	309	−46	−202	−1	6	−64
67	27.8	31.9	31.8	315	−46	−216	1	−3	−49
68	32.4	33.6	33.4	328	−47	−287	1	2	2

* T_A, T_W, and T_S, air temperature, mean radiant wall temperature, and mean skin temperature.

† M, metabolism.

‡ W, work, as measured from the calibration of the ergometer.

§ E, evaporative heat loss.

¶ Computed on assumption that radiation area is 70 per cent of total body area.

‖ Computed from changes in skin temperature and rectal temperature.

** Computed from $M - W - E \pm S \pm R$.

vection loss, computed from the algebraic sum of metabolism, work, evaporation, storage, and radiation was chiefly determined by the large measured factors of metabolism and evaporation.

In the upper part of figure 1 we have plotted the convection loss, as thus estimated, against the difference between mean skin temperature and air temperature for each experiment. The slope of this curve gives a convection constant of between 10 and 11 kgm.-cal. of heat loss for each °C. difference in temperature between skin and air. It will be noted that the points fall about the mean slope as drawn except in the case of one of the

two coldest experiments for subject VII. This departure is in accord with our earlier studies which show that under cold conditions considerable storage (cooling of body tissues) occurs without being immediately mirrored in fall of skin and rectal temperature.

When the convection constant is computed for the individual as a unit, it is apparently the same for both subjects. If computed per unit surface area—as in our earlier work on subjects at rest—the constant is 5.3 for subject VII (a large individual) and 6.7 for subject IX (a small individual). Since, in the case of a subject actively pedalling on a bicycle, convection loss is largely determined by the movement of the legs it is not surprising that, when subjects differ in size, the over-all heat loss from the whole body gives more consistent results than that computed for a standard area. In a subject at rest, both metabolism and processes of heat loss are adjusted to total surface area but this is not the case in exercise where the level of heat production is arbitrarily set by the severity of the task. We shall, therefore, present our data in general on the basis of the total subject and not—as in much of our previous work—per square meter of body surface.

Influence of pedalling rate and air movement on the convection constant. For rough comparison with results on resting subjects we may take a convection constant of 5.9 per square meter per hour (per °C. difference in temperature between skin and air) as representing an approximate mean value for the two subjects under working conditions.

In our earlier work on resting subjects in a semi-reclining position we found the convection constant to be 1.0 $\sqrt{V}$ where V is the velocity of air movement in centimeters per second (Winslow, Gagge and Herrington, 1940). In the present series of studies four tests were made on each subject in a sitting posture on the bicycle but at rest without pedalling. The constants obtained are shown in table 2, for the whole subject, and per square meter, and also the component which gives the constant when divided by $\sqrt{V}$.

The mean of these eight values is 0.8 $\sqrt{V}$ which means that the rate of convection loss for the subject sitting up at rest is slightly less than in the case of the reclining subject. This is in accord with what we would expect. The reclining subject has his long diameter in a more nearly horizontal position than the sitting subject; and the more nearly vertical is the position of the heated cylinder, the less will be the rate of convection loss.

It will be noted that the constants for convection loss from the whole body of the subject in table 2 for low air velocities are less than one-third those derived from table 1. The difference is obviously due to increased convection due to the movements (particularly of the legs) involved in pedalling. To measure this factor and to determine its relation

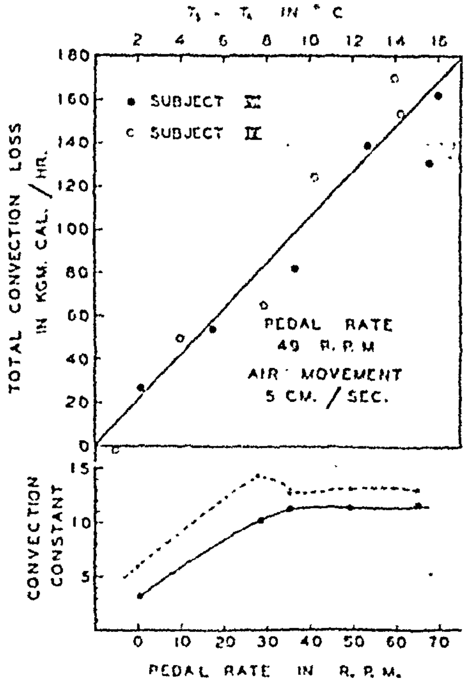

Fig. 1

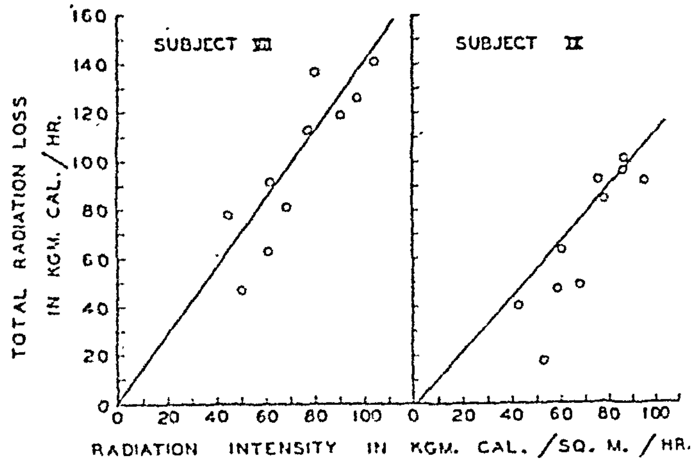

Fig. 2

Fig. 1. Upper graph. Relation of heat loss by convection to difference between skin and air temperatures (basic curve for determination of convection constant).

Lower graph. Relation of convection constant to varying pedalling rates.

Fig. 2. Relation of heat loss by radiation to intensity of radiative demand of the environment (basic curve for determination of radiation area).

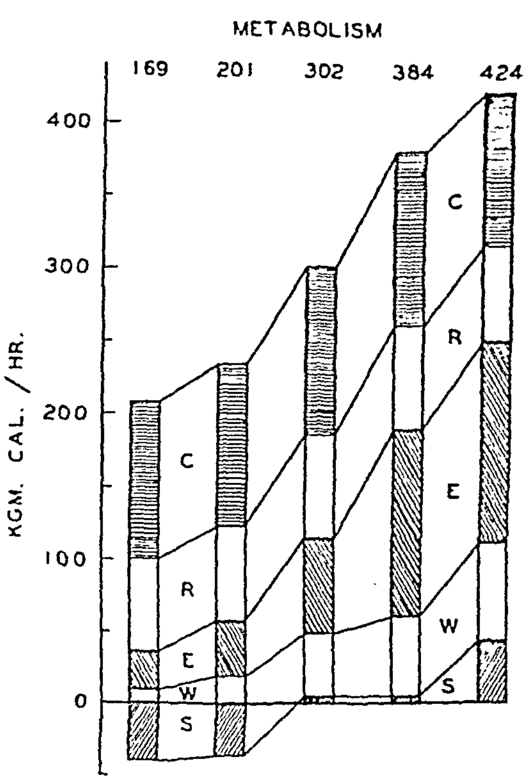

Fig. 3

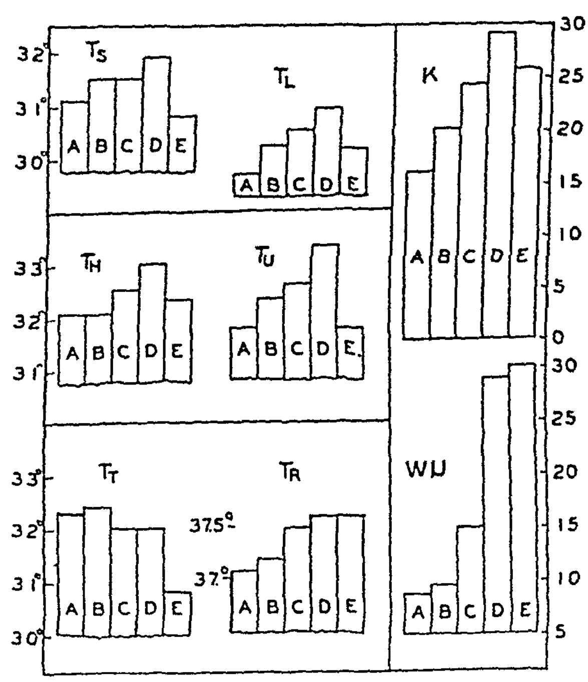

Fig. 4

Fig. 3. Variation in heat interchange by various avenues with rate of metabolism (as determined by varying rate of work).

C = convection heat loss, R = radiation heat loss, E = evaporative heat loss, W = energy consumed in work, S = storage (values below zero denote body cooling). Total height of column equals metabolism.

Fig. 4. General and regional skin temperatures, conductance, rectal temperatures, and wetted area for five different rates of metabolism. Metabolic value: A, 169; B, 201; C, 302; D, 384; E, 424 kgm.-cal. per hour.

to air movement we conducted 50 experiments, with varying pedalling rate, 30 at normal low air velocities and 20 with high air velocities (17–23 cm./sec.). The total amount of work performed (and the related metabolism) were approximately the same, the different pedalling rates being

TABLE 2

Convection constants for subjects at rest in sitting posture on the bicycle

AIR VEL.	SUBJECT VII			SUBJECT IX		
	$C/\Delta T$	$\frac{C}{\text{sq. M} - \Delta T}$	$\frac{C}{\text{sq. M} - \Delta T - \sqrt{V}}$	$C/\Delta T$	$\frac{C}{\text{sq. M} - \Delta T}$	$\frac{C}{\text{sq. M} - \Delta T - \sqrt{V}}$
cm./sec.						
4.1	2.5	1.2	0.6	3.2	2.0	1.0
5.1				3.6	2.3	1.0
6.1	3.4	1.7	0.7			
17.8	7.4	3.7	0.9	5.0	3.1	0.7
19.3	5.6	2.8	0.6			
20.3				5.5	3.4	0.8

TABLE 3

Total convection constant for varying pedalling rates at low and high air movement

SUBJECT	AIR MOVEMENT									
	4-8 cm./sec.					17-23 cm./sec.				
	Pedalling rate, R.P.M.									
	0	28-29	34-36	48-50	63-68	0	28-29	34-36	48-50	63-68
VII	2.5	10.3	11.2	17.8	13.1	7.4	15.3	13.4	18.1	13.0
	3.4	10.8	11.2	9.6	11.9	5.6			13.8	
		11.4	11.8	11.7	12.0				10.3	
				12.2					17.1	
				11.5						
				11.4						
IX	3.2	7.2	12.7	9.7	11.8	5.0	16.6	12.6	13.6	12.4
	3.6	10.8	10.1	10.1	11.5	5.5	14.4	12.2	9.4	13.4
		11.5	11.0	10.4	9.7		11.4	12.6	9.2	14.1
				13.5					12.9	
				9.2						
				10.8						
Mean.........	3.2	10.3	11.3	11.5	11.7	5.9	14.4	12.7	13.1	13.2

regulated by decreasing or increasing the resistance of the bicycle. We have expressed all results in terms of total body area since—as pointed out above—this is the most consistent measure of convection loss under the conditions of the experiment. We have also added one column of table 2 for comparison.

It will be noted (table 3) that the process of pedalling even at the low

rate of 28 to 29 revolutions per minute, nearly tripled the convection constant and further increase did not materially change the picture. The lack of further increase with more rapid pedalling is perhaps not surprising, since the legs carry a blanket of warmed air with them and movement at more than a certain rate might naturally be ineffective.

The mean results of table 2 plotted at the bottom of figure 1 confirm our constant of 11 for the pedalling subject at low air movement; and this constant for an air movement of about 5 cm. per sec. is equivalent to the constant which would be observed for a subject at rest with an air movement of 30 to 40 cm. per sec. This latter factor is an equivalent measure of the increase in convective heat loss due to the movements of the body.

So far as the influence of air movement itself is concerned, we note that an increase from 5 cm. to 20 cm. for the resting subject doubled the convection loss which accords with the square root formula.

In the case of the pedalling subject, a similar increase in external air movement only raised the convection constant from 11 to 13. If, however, we recall that the actual combination of air movement about the body and body movement through the air is 30 + 5 in the first case, and 30 + 20 in the second case, the convection constant should be raised a little less than 20 per cent by the additional air movement—as is the case. For our standard pedalling rate, the convection constant is about 11, and this value (rather than the 10.5 indicated in the upper part of the graph) has been used in our later computations.

Determination of the radiation area. Having determined the convection constant, a series of 10 experiments were made with each of our two subjects to determine their radiation area. The experiments were so set up that air temperature varied but little (from 18.4 to 22.4°C.) and that storage (estimated from change in skin and rectal temperature) was small (from +20 to −11 kgm.-cal.) in relation to total heat interchange. Skin temperature changed less than 1°C. (except in three cases, where the changes were less than 1.2°, respectively). Rectal temperatures changed less than 0.3° (except for one case where it reached 0.55°). Metabolism varied from 347 to 374 for subject VII and from 319 to 343 kgm.-cal. for subject IX. The work performed varied from 48 to 55 kgm.-cal.

It will be noted from table 4 that with both subjects, radiation loss dropped rapidly between experiment 12 and experiment 13, when wall temperatures began to exceed 15°C. In the case of subject VII, active increase in sweat secretion took place from experiment 8 on, while the increase began with subject IX in experiment 11 when the radiant temperature reached about 14°C. Clearly, the subjects were in the phase of evaporative regulation throughout all these experiments, the increasing sweat secretion just balancing a tendency to decreased radiation as conditions became progressively warmer.

For determination of the radiation area, the total radiation loss for each subject has been plotted in figure 2 against "radiation intensity"—the heat loss per square meter which would be expected from Stefan's Radiation Law for the recorded difference between skin temperature (T_S) and mean radiant temperature of the surroundings (T_W). The slope of the graphs gives the radiation area which amounts to 72 per cent of the total

TABLE 4

Experiment conducted for the determination of the radiation area

SUBJECT AND EXPERIMENT	TEMPERATURE, °C.			HEAT INTERCHANGE, KILOGRAM-CALORIES PER HOUR						RELATION INTENSITY, R‡
	T_A	T_W	T_S	M	W	E	C	S*	R†	
VII- 8	18.5	8.6	29.7	350	−50	− 35	−119	−5	−141	−104
9	18.4	10.8	30.3	347	−50	−53	−129	+11	−126	−97
10	18.9	12.3	30.2	351	−51	−68	−120	+7	−119	−90
11	19.9	14.3	30.1	357	−51	−52	−108	−9	−137	−80
12	19.8	15.2	30.3	372	−52	−100	−111	+8	−113	−77
13	20.6	18.0	31.4	366	−55	−118	−114	+8	−81	−67
14	21.1	19.4	31.4	350	−50	−104	−109	+5	−92	−62
15	21.3	20.4	31.9	374	−51	−141	−112	−7	−63	−61
16	22.4	22.6	31.9	351	−51	−145	−101	−7	−47	−50
17	22.3	23.1	31.6	358	−48	−143	−99	+10	−78	−45
IX- 8	18.9	9.3	28.8	330	−51	−83	−105	+1	−92	−96
9	19.3	11.5	29.2	328	−48	−77	−105	+3	−101	−88
10	19.3	11.7	29.2	330	−50	−70	−105	−9	−96	−87
11	19.6	13.8	29.5	343	−50	−100	−105	−4	−84	−79
12	19.9	15.1	30.0	340	−51	−101	−107	+11	−92	−76
13	20.2	16.8	30.1	331	−50	−142	−105	+15	−48	−68
14	20.4	17.9	29.7	328	−53	−122	−98	+8	−63	−61
15	20.6	19.1	30.4	331	−50	−150	−104	+20	−47	−59
16	21.4	20.6	30.7	319	−50	−155	−98	+1	−17	−53
17	22.4	22.7	30.9	337	−50	−159	−90	+2	−40	−43

* Computed from changes in skin temperature and rectal temperature.

† Computed from $M - W - E \pm S - C$.

‡ Calculated from Stefan's Radiation Law,

$$R = 4.92 \times 10^{-8} (T_W^4 - T_S^4).$$

surface area for subject VII and 67 per cent for subject IX. It will be noted that under hot conditions for subject IX, three of the points fall below the graph which—as shown in our earlier communications—indicates that the sweat is running off without evaporating and thus is not exerting its cooling effect upon the body.

It appears then that the effective radiation area for a subject pedalling on a stationary bicycle is about 70 per cent of the total surface area, as was found to be the case for the resting subject (Winslow, Gagge and Herrington, 1940).

In all future computations of the present paper, where the pedalling rate is 48, we shall use the convection constant of 11, and compute radiation as a 70 per cent radiation area, estimating storage by difference from the formula

$$M - W - E \pm C \pm R = S.$$

The influence of variations in work and metabolism upon thermal interchange. The major difference between the conditions here discussed (aside from the influence of body movement upon the convection constant) is the performance of physical work and the accompanying higher metabolism. A series of eight experiments were conducted with each of our two subjects in which the amount of work performed was the only variable, to measure the influence of these factors more closely.

In these experiments, air and wall temperatures were both held between 20.6 and 21.6°C. Under these conditions the mean skin temperatures of the subjects varied between 30.3 and 32.6°C. Subject VII had a skin temperature between 31.1 and 31.6°C. in all but two of the high-work experiments in which T_S rose to 32.6°. Subject IX had a T_S varying from 30.3 to 31.7°C., corresponding to his higher evaporative heat loss. The data with regard to thermal interchange are summarized in table 5.

It will be noted that when performing work at a rate of 8 kgm.-cal. per hour, subject VII had a metabolism of about 200, compared with a resting figure for this subject while on the bicycle of 140; subject IX had a metabolism of about 150, compared with a resting figure of 110. When performing work of 19 kgm.-cal., the metabolism for subject VII changed very slightly while for subject IX it rose to nearly 200. At a work-rate of 45 kgm.-cal. both subjects showed a metabolism of about 300. At 57 kgm.-cal., both metabolisms rose to nearly 400; and at 70 kgm.-cal. of work to over 400. Omitting experiment VII-62 where metabolism was abnormally high, the efficiency of the work performed varied between 0.20 and 0.30 and averaged 0.24.

Convection and radiation showed no significant relation to work as must necessarily be the case since skin temperatures remained so nearly uniform. Storage was positive (cooling of body tissues) with work rates of 8 and 19 kgm.-cal. It was negative at higher work rates, except in experiments VII-63 and IX-51.

Since no great difference in reaction was manifested by the two subjects and since the number of experiments with each subject was the same, we have averaged all results for both subjects at a given work rate and presented them in figure 3. Positive storage (cooling of body tissues) has been plotted below the base-line, negative storage, above. The total height of the combined bars above the base line corresponds to the metabolism.

It will be noted that as work (and metabolism) increased, evaporative heat loss increased *pari passu* but not at so great a rate (except for the interval between 45 and 57 kgm.-cal. where evaporation almost exactly kept up with the increased thermal load); therefore, storage passes gradually from a positive to a negative value.

It is of interest to note how fallacious is the habit of expressing heat-interchange by various avenues as percentages of metabolic heat production. With subjects at rest (and equal wall and air temperatures) radiation and convection heat losses are nearly equal. With a pedalling subject, convection loss is about 70 per cent in excess of radiation loss as a result

TABLE 5

Thermal interchange with varying work loads and related metabolism (pedalling rate held constant at 38 R.P.M.)

SUBJECT AND EXPERIMENT	TEMPERATURE, °C.			HEAT INTERCHANGE, KILOGRAM-CALORIES PER HOUR					
	T_A	T_W	T_S	M	W	E	R	C	S
VII-62	21.1	21.4	31.6	210	−8	−29	−76	−116	19
66	21.5	21.6	31.2	169	−8	−26	−72	−107	44
63	20.8	21.3	31.5	211	−19	−29	−76	−118	31
67	21.3	21.5	31.1	203	−19	−40	−71	−108	35
64	21.2	21.4	32.6	295	−45	−59	−83	−125	17
68	20.9	21.1	31.3	300	−45	−42	−76	−114	−23
65	21.2	21.3	32.6	397	−57	−92	−84	−125	−39
69	21.1	21.2	31.3	439	−70	−105	−75	−112	−77
IX-48	21.0	21.3	30.8	144	−8	−27	−56	−108	55
52	21.4	21.6	30.6	153	−8	−34	−53	−101	43
49	21.1	21.4	31.7	201	−19	−38	−61	−117	34
53	21.1	21.4	31.5	191	−19	−43	−59	−114	44
50	20.6	21.1	31.4	317	−45	−86	−61	−119	−6
54	20.9	21.3	30.8	297	−45	−82	−56	−109	−5
51	20.9	21.2	31.0	371	−57	−169	−58	−111	24
55	21.0	21.1	30.3	408	−70	−172	−54	−102	−10

of the movement of the limbs. Under such conditions, air temperature is much more important than mean radiant temperature in determining comfort. Furthermore, as seen from table 5, evaporative heat loss increases threefold for subject VII with increasing work and more than five times with subject IX, while convection and radiation losses remain fairly constant.

Reactions of working subjects at various operative temperatures. The operative temperature has been defined in our earlier studies as the temperature representing the combined influence of air and surrounding surfaces on heat losses from the body. The actual air temperature and mean radiant temperature must be duly weighted for radiation and convection

constants, respectively. To avoid this computation, we have conducted 19 experiments for each subject in which air temperature and mean ra-

TABLE 6

*Basic data at various operative temperatures with wall and air temperature equal**

SUBJECT AND EXPERIMENT	DATE	T_O†	T_S†	T_H†	T_U†	T_T†	T_L†	T_R†	M‡	W‡	E‡	R‡	C‡	S‡	K§	W¶	VOTE‖
VII-72	1/15/41	12.4	27.9	29.4	28.0	29.3	26.4	37.4	305	−47	−26	−109	−171	46	15.8	5.6	1
71	1/14/41	15.5	29.6	30.6	31.1	30.8	27.6	37.5	328	−46	−45	−102	−155	20	18.9	9.5	2
34	9/10/40	15.6	30.4	30.0	31.3	31.6	29.1	37.6	320	−45	−45	−108	−162	40	21.6	10.3	4
33	9/ 6/40	15.7	29.9	30.1	30.4	31.2	28.6	37.2	313	−46	−38	−103	−155	29	20.0	8.5	2
47	11/ 1/40	15.8	29.8	31.1	31.0	31.2	28.0	37.9	335	−46	−48	−100	−155	14	18.5	10.4	2
46	10/31/40	16.1	30.0	31.3	30.8	31.8	28.1	37.7	315	−46	−46	−100	−153	30	19.2	9.6	3
70	1/13/41	18.7	31.2	31.6	33.4	31.7	29.6	37.4	303	−46	−45	−93	−135	16	21.8	8.2	3.5
50	11/ 7/40	20.2	31.2	31.8	32.9	31.9	29.7	37.6	317	−46	−49	−82	−109	−31	18.5	9.4	4
73	1/16/41	20.8	31.8	32.8	33.1	32.6	30.5	37.9	314	−47	−48	−82	−122	−15	18.7	10.6	4.5
15	7/16/40	20.9	31.9	31.9	32.6	32.5	30.8	37.7	374	−51	−141	−86	−117	−21	21.9	30.0	4.5
68	1/ 7/41	21.0	31.3	32.2	32.2	31.6	30.4	37.4	300	−45	−42	−76	−114	−23	18.7	8.0	3.5
57	11/27/40	21.2	31.8	32.3	33.2	31.9	30.8	37.7	380	−46	−54	−77	−120	−83	24.4	10.1	4
64	12/19/40	21.3	32.6	33.2	33.7	32.8	31.6	38.0	295	−45	−59	−84	−125	18	24.4	10.5	4.5
16	7/17/40	22.5	31.9	32.1	32.7	32.8	30.7	37.7	351	−51	−145	−70	−105	20	27.2	30.0	4
17	7/18/40	22.7	31.6	31.6	32.6	32.0	31.0	37.5	358	−48	−143	−64	−102	1	26.0	27.6	5
74	1/17/41	24.3	33.4	33.9	34.4	34.1	32.3	37.7	307	−46	−85	−71	−99	6	30.7	14.5	4.5
75	1/20/41	26.8	33.4	33.6	34.2	33.7	32.9	37.7	317	−46	−117	−51	−74	−29	27.6	20.8	4.5
76	1/21/41	31.6	34.7	35.2	35.0	34.6	34.4	37.8	320	−46	−200	−25	−34	−15	41.3	33.8	4.5
6	2/ 5/41	33.2	34.3	35.1	34.6	34.2	34.0	37.6	316	−46	−234	−0	−24	−12	38.2	40.2	5
IX-58	1/15/41	12.4	28.4	30.5	30.1	31.2	25.2	37.8	310	−46	−44	−90	−176	46	20.6	11.8	2
57	1/14/41	15.7	29.4	31.1	31.0	31.6	26.8	37.4	307	−47	−63	−79	−151	33	22.9	16.3	3
33	11/ 1/40	15.8	29.6	31.3	30.9	31.6	26.9	37.7	303	−46	−48	−78	−153	22	21.5	13.3	3
23	10/31/40	16.1	29.8	31.9	30.6	31.8	27.5	37.9	316	−46	−49	−78	−151	8	21.5	13.5	3
32	9/10/40	15.6	29.7	30.8	30.7	31.6	27.6	37.3	288	−46	−49	−81	−154	42	23.4	15.1	3
22	5/ 9/40	16.2	30.2	31.4	31.1	31.7	28.3	37.4	322	−46	−69	−82	−152	27	26.2	19.0	4
56	1/13/41	18.4	31.3	32.4	32.2	32.3	29.7	37.7	310	−46	−55	−76	−140	7	26.5	12.7	4
15	7/18/40	19.9	30.4	32.3	31.8	31.3	30.5	37.9	331	−50	−150	−66	−108	43	27.0	46.3	4
30	11/ 7/40	20.2	31.3	33.1	32.5	32.5	29.6	37.8	329	−46	−76	−66	−120	−21	25.3	18.3	4.5
59	1/16/41	20.6	31.0	32.9	32.7	32.0	29.1	37.6	329	−46	−101	−61	−116	−5	26.4	23.8	4
50	12/19/40	20.9	31.4	32.4	32.6	32.2	30.2	37.4	317	−45	−86	−61	−119	−6	27.7	20.8	4
16	7/17/40	21.0	30.7	32.0	31.6	31.8	29.3	37.4	319	−50	−155	−59	−102	47	29.4	46.0	4
54	1/ 7/41	21.1	30.8	32.1	31.8	31.4	29.6	37.2	297	−45	−82	−56	−109	−5	24.1	20.3	4
44	11/27/40	21.3	30.9	32.2	32.3	31.9	29.2	37.5	308	−46	−117	−54	−110	19	26.7	28.9	4
17	7/18/40	22.6	30.9	32.9	32.1	31.4	29.5	37.8	337	−50	−159	−49	−94	15	27.2	45.3	4.5
60	1/17/41	23.9	31.7	33.4	33.2	32.1	30.4	39.6	335	−46	−158	−47	−87	3	30.9	38.8	4
61	1/20/41	26.4	31.8	33.3	32.9	31.9	30.8	37.5	298	−46	−187	−31	−63	29	31.0	40.0	4
62	1/21/41	29.4	32.9	34.1	33.3	31.8	31.9	37.6	323	−46	−232	−22	−40	17	39.2	54.5	4.5
6	2/ 5/41	33.0	33.4	35.3	34.2	32.4	33.9	37.7	328	−47	−287	1	−11	16	43.3	67.0	5

* Relative humidity, 40–50 per cent saturation; air velocity, 5–8 cm. per sec.; pedalling rate, 48 R.P.M.

† Operative temperature, mean skin temperature, temperatures of head, upper extremities, trunk and lower extremities, rectal temperature, °C.

‡ Metabolism, work, evaporative heat loss, radiation loss, convection loss, storage.

§ Conductance in kilogram-calories per square meter per hour per °C. difference between T_R and T_S.

¶ Degree of wettedness, in kilogram-calories per square meter per hour per centimeter of Hg vapor pressure difference between skin and air.

‖ Vote of subject as to thermal comfort on a scale of 5, 1 being cold and 5 hot.

diant temperature were nearly identical, almost never differing by more than 1°C., and at a constant air movement of 6 to 8 cm. per sec. Under

these particular circumstances, the mean of the two temperatures is, for all practical purposes, the operative temperature itself; and the pertinent data for these 38 experiments are presented in table 6. In all instances, relative humidity was moderate (50–60 per cent) and the work done (at a standard pedalling rate of 48–50 revolutions per minute) varied only from 45 to 51 kgm.-cal. per hour.

Metabolism and storage. It will be noted from table 6 that metabolism was generally between 290 and 340 kgm.-cal. for both subjects. Subject IX showed one low value of 288 and a maximum value of 335 kgm.-cal. Subject VII had a minimum value of 295 and four abnormally high values of 351, 358, 374 and 380, respectively. With neither subject, however, was there any relation between metabolism and operative temperature.

Storage values were also fairly uniform, ranging from −31 (heating of the body tissues) to +50 (cooling of the body tissues) in all but one instance. In one experiment with subject VII (a pyknic subject) a very high value for negative storage of −83 was recorded. This was in experiment VII-59, which was the experiment with a metabolism of 380. Omitting this single abnormal figure we find that mean storage values varied as below.

	To		
	Under 17°	18°–22°	Over 22°
Storage, Subject VII	+33	−10	−5
Subject IX	+30	+9	+16

Thus, for both subjects, body cooling was definitely present in every experiment at all operative temperatures below 17°C. At operative temperatures of 18° and above, subject VII (the pyknic) showed a general tendency at end of experiment toward body heating during exercise, subject IX (a slender subject) a slight tendency to body cooling.

The mean, semi-reclining metabolism of these two subjects is about 86 Calories, so that mean work metabolism here is about 3.7 resting basals. At 31°C. we have usually said it takes 4°C. to dissipate body heat: 3.7 × 4° equals 14.8°; 31° minus 14.8° equals 16.2°, which is very close to our 17°C. neutral condition. It appears as though the equilibrium point for a given work-rate might almost be computed by multiplying the working metabolism expressed as a multiple of basal metabolism by 4 and subtracting the value obtained from 31°.

Heat loss by radiation and convection. Heat loss by both radiation and convection, of course, steadily increased with falling operative temperature. It is interesting to note from table 6 that convection loss for the two subjects was essentially identical at any given operative temperature.

For any given operative temperature, however, subject VII lost about 20 more kgm.-cal. than subject IX by radiation. This, of course, merely illustrates the fact that under working conditions—where the rapid movement of the legs is the main factor in convection loss—body build and body size have little influence on the magnitude of the convection but they still play an important rôle in determining the magnitude of the radiation loss.

Skin temperature, rectal temperature and conductance. It will be noted, from table 6, that mean skin temperature for both subjects falls with decreasing operative temperatures but a trifle more rapidly for subject VII, the pyknic subject, because his skin temperature was higher under hot conditions. Thus, for subject VII the mean T_S fell from 34.3° at a T_O of 33.2° to a value of 27.9° at a T_O of 12.4°. For subject IX, the corresponding T_O values were 33.4° and 28.4°. In each case a decrease of 20° in operative temperature caused a fall of from 5° to 6.5° in mean skin temperature. This is much the same reaction as that of the resting subject (Herrington, Winslow and Gagge, 1937).

For comparison, we have averaged the data for each half-degree temperature interval and have compared the results, in figure 5, with those obtained for subjects IX and VII in a resting position as reported in an earlier paper (Winslow, Herrington and Gagge, 1937) for both mean skin temperature and conductance.

It is most interesting to note that the variations of both skin temperature and conductance with falling operative temperature are almost identical for the resting and the working subject. Each individual has his own level of skin temperature under warm conditions, that for subject VII being high and for subject IX being low. Under cold conditions all fall close together. With subject VII—observed in both series—skin temperature and conductance (which varies with vasodilatation) were actually lower at a given high operative temperature under work conditions than at rest. This indicates a surprisingly perfect adaptation to the enormously increased load of heat production under conditions of vigorous physical work.

Furthermore, the fall of skin temperature for the main regions of the body surface was much the same in the working and resting state. We had thought that, on account of the large amount of heat produced in the lower extremities, the skin temperatures of this region might fall less rapidly under working conditions; but such was apparently not the case.

It will be noted that, in general, the regional skin temperatures for the resting subjects were considerably higher than for the working subjects (the only exception being that of the upper extremities under cool conditions). This is, of course, due to the higher evaporative heat loss of the working subjects. It may be noted that under very hot conditions the skin tem-

peratures of the resting subjects dip sharply down as their sweat secretion sets in. The individual regions of the body exhibit the same general trends in all cases.

Rectal temperature, on the other hand, shows a marked difference between the working subject and the resting subject, as indicated in figure 5.

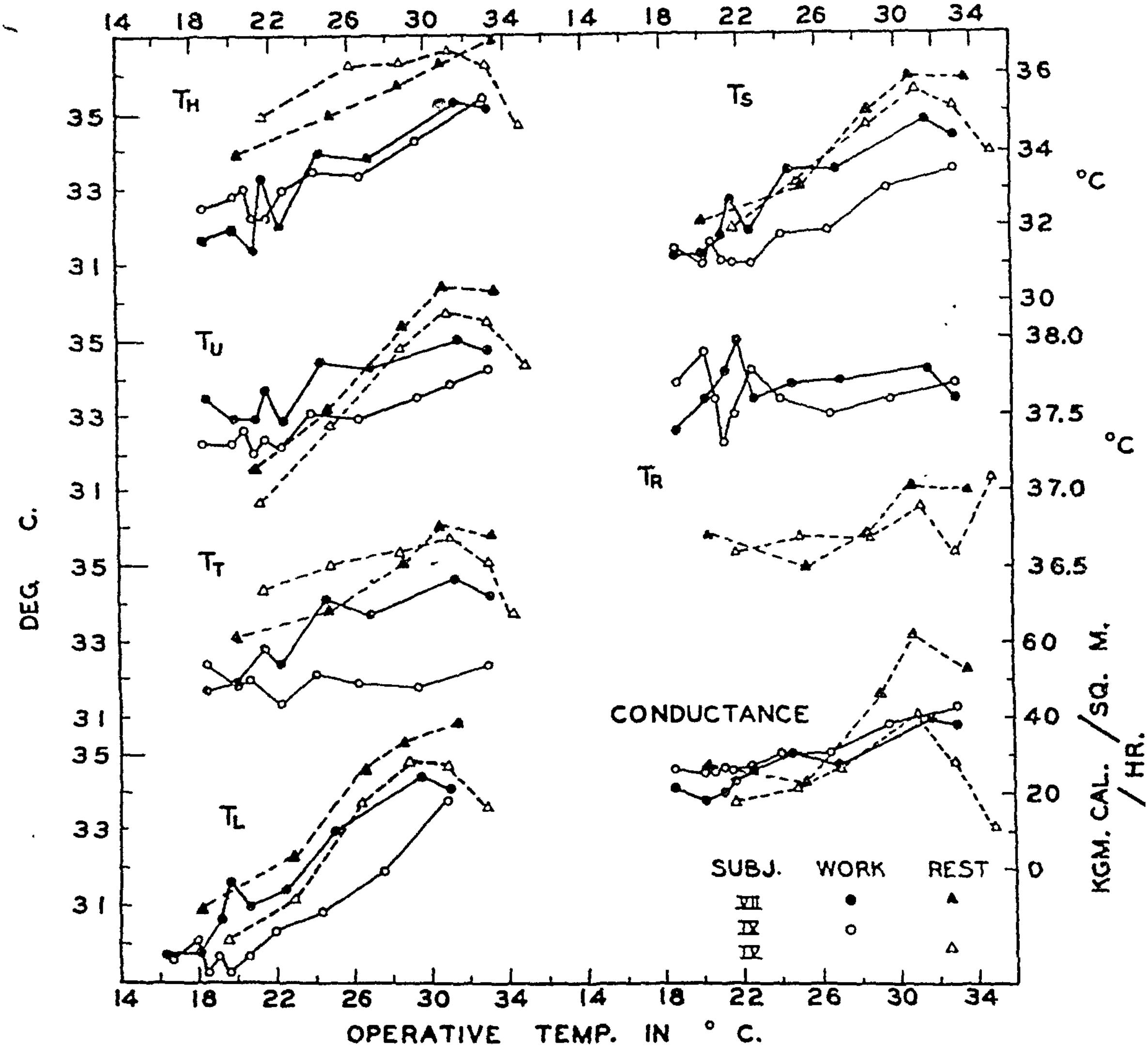

Fig. 5. Relation of general and regional skin temperatures, conductance, and rectal temperatures to operative temperatures.

For resting subjects, the rectal temperature is in the neighborhood of 36.9° at operative temperatures above 30° and falls to about 36.6° in a cooler environment. The rectal temperature of the working subjects, however, remains at about 37.6° throughout the whole range of operative temperatures. A difference of 1° in rectal temperature is highly significant and is no doubt associated with the high metabolism of the working subject.

Evaporation and wetted area. The evaporative heat loss clearly represents the mode of thermal adaptation to working conditions and the results in this respect are, therefore, of special interest. It will be noted from table 6 that at operative temperatures below 19°, evaporative heat loss is always relatively low (below 70 kgm.-cal.). Between 19° and 25° T_O it varies very widely (from 40 to 160) and apparently at random. Above 25° it is always high and rises steadily with further rises in T_O.

The factor which governs the adaptive process of evaporation (Gagge, 1937) is the degree of wettedness of the skin surface (w_μ). This factor again shows low values (under 20) below 19° T_O, and high and progressively increasing values above 25° T_O—with wide variations between 20° and 25°. We were puzzled at first by the sharp and apparently meaningless variations in the middle range—until it was noted that the low values had always occurred in winter, high values in summer. In the light of earlier studies (Winslow, Herrington and Gagge, 1938) which showed a seasonal variation in the efficiency of the evaporative mechanism this seemed to offer a reasonable explanation of the apparent discrepancies. In figure 6 we have, therefore, plotted separately the data for each subject at each season—with earlier data for resting subjects for comparison.

It is clear from this graph that with both subjects VII and IX in winter the process of active sweat secretion in the working subject sets in at about 20° T_O. In summer, both subjects react more promptly (somewhere between 16° and 20°) and at 20° have reached a fairly high level of evaporative wettedness. For the resting subject, the onset of evaporative regulation is reached only above 30°. At all points, subject IX (the leptosome) exhibits a higher evaporative rate than subject VII. This is, no doubt, because the slender subject, when working at the same high metabolic rate as the stout subject, must balance the higher radiation loss of the latter.

Sensations of comfort. At the close of each experiment, the subjects expressed their temperature sensations on a scale in which 1 represented "cold," 2, "cool," 3, "ideal," 4, "warm," and 5, "hot."

At operativé temperatures below 15°C. the mean vote of both subjects was 1.5; at temperatures between 15° and 17° it was 2.9; at temperatures between 17° and 22°—and also at temperatures above 22°—the mean vote was 4.1. Thus, the ideal sensation for the working subject was at about 16°C., as compared with 28° for the resting subject (Winslow, Herrington and Gagge, 1937). On the other hand, the sensation of thermal comfort for the working subject occurs at a slightly lower skin temperature (31.3°) than for the resting subject (32.8°). Thus, it appears that an increase of metabolism from 100 to 300 kilogram-calories is equivalent to a difference of 12° in operative temperature, so far as its influence on comfort is concerned.

Reactions of subjects working at various rates. To check up on the observed phenomena from a slightly different angle, we may analyze physiological reactions for two series of experiments with each subject under

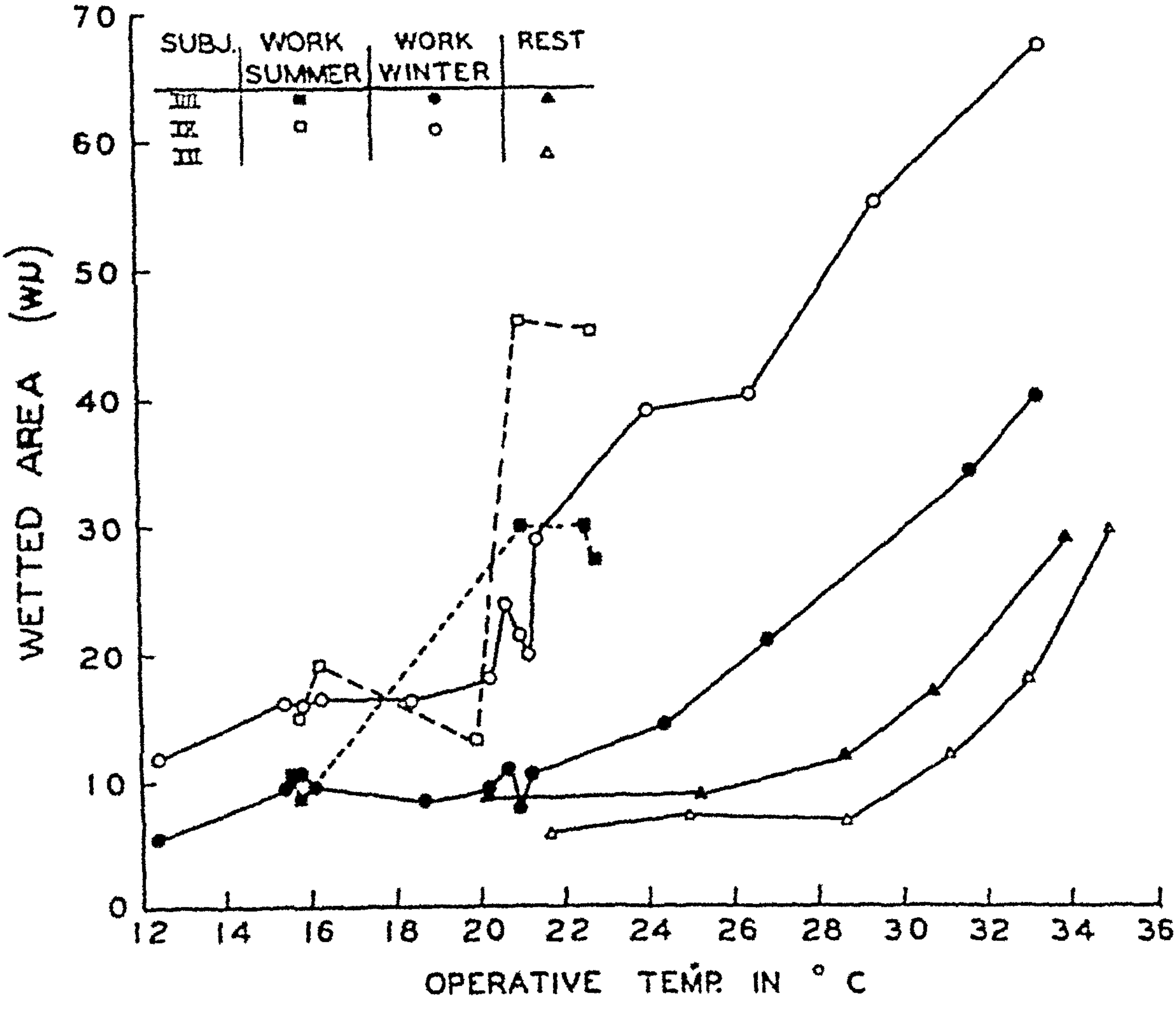

Fig. 6. Relation of wetted area to operative temperatures for resting subjects and for working subjects in summer and in winter.

standard atmospheric conditions (T_o, 21°C.) pedalling on the bicycle at a fixed rate (35 revolutions per minute) at five different loads, for which basic physiological data have been presented in table 5. We have arranged results for the two subjects for the following variations in work and metabolism.

	A	B	C	D	E
Work	8	19	45	57	70
Metabolism, mean	168	292	302	384	424

We present in figure 4 the mean values for four experiments at each of the three lower work rates and two experiments at each of the higher work rates, with respect to regional skin temperatures, rectal temperature, conductance and degree of wettedness.

It will be noted that the skin temperatures of head, upper extremities and lower extremities all rise slightly with increasing metabolic activity up to the fifth and highest metabolic rate, at which rate these temperatures fall slightly, apparently as a result of over-compensation due to very high evaporation. Trunk temperatures tend rather to fall with increased metabolic activity, very rapidly at the highest metabolic rate. This phenomenon is undoubtedly related to the fact that this is the area of the body where evaporative cooling is chiefly localized. Rectal temperature rises steadily with increasing metabolism. Conductance rises (except at the fifth work rate) and sweat secretion (w_μ) shows very great and progressive increase at metabolic activities above 200 kgm.-cal.

SUMMARY OF CONCLUSIONS

Study of the reactions of two unclothed male subjects pedalling on a stationary bicycle at such a rate as to increase their metabolism to over 300 kgm.-cal. per hour revealed the following differences, as compared to earlier results obtained with similar subjects at rest in a semi-reclining position (with a metabolism of 80–100 kgm.-cal.).

1. The radiation area is the same as that for the resting subject, approximately 70 per cent of the body surface area.

2. The convection constant for a subject sitting on the stationary bicycle but at rest is about 20 per cent less than that for the resting reclining subject, as a result of his more nearly vertical position. When the subject is pedalling at a rate of some 30 revolutions per minute, the convection constant is greatly increased; and this increase (due to the movements of the body and particularly of the legs) is equivalent in cooling power to an air movement of 30 to 40 cm. per sec. Under these conditions, the general body build of the subject has little influence on convection loss. Further increase in pedalling rate does not change the constant materially. Air movement produces its usual influence, but since this effect of air (or body) movement varies with the square root of velocity, the actual effect of even rather high air movements (superimposed on that of body movements) is relatively slight.

3. Increased sweat secretion, with consequent evaporative cooling, balances very perfectly the increased heat which must be dissipated as a result of high metabolism. The rate of sweat secretion increases three to five-fold for a two to three-fold rise in metabolism. Active sweat secretion sets in under conditions of winter adaptation at an operative temperature of 20° instead of at 30° with the resting subject. In summer the trigger

mechanism of sweat secretion is more sensitive and initiates active sweat secretion somewhere between 16° and 20°. At 21°C. T_O, the thermal reactions of the unclothed body are in balance with a metabolism of 350 kgm.-cal.

4. Evaporative regulation in the case of the working subject operates so effectively that skin temperature is held remarkably constant, actually being lower—at a given T_O—for the working than for the resting body. With increasing metabolism at a given T_O, the skin temperature of the trunk actually falls. Rectal temperature, however, rises appreciably under working conditions. It would seem, under these circumstances, clear that internal temperature—not skin temperature—must control the sweat-secreting mechanism.

5. A thermal sensation of pleasantness is produced in the unclothed working subject (with a metabolism of 300–400 kgm.-cal.) at a T_O of 16°, instead of 28° with the resting subject. In both instances, maximum comfort is experienced about 2° below the point where active sweat secretion begins.

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RESPIRATORY AND CIRCULATORY RESPONSES TO ACUTE CARBON MONOXIDE POISONING

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Asmussen and Chiodi (1) have reported that CO saturations ranging from 23 to 33 per cent have but slight effects on the circulatory and respiratory systems of man. The possibility that higher saturations with CO would lead to responses differing in type or degree led us to extend these studies. Observations have been made on pulmonary ventilation, cardiac output and plasma pH in man and dog. The effect of CO on the respiratory response to high CO_2 tension has been studied in man.

Haldane (7) was one of the first to study by self-experiment the effects of CO poisoning. His report, that a hyperventilation began at about 35 per cent saturation, is not in harmony with the clinical observations of Sayers et al. (13) and of Hayhurst (8) that CO poisoning does not influence respiration. However, Haggard and Henderson (6) found that in dogs 50 per cent or more saturated with CO there was sufficient hyperventilation to induce an alkalosis and a decreased blood alkali. Sectioning of the vagi prevented both these responses. Theil (16) also noted a hyperventilation in dogs but his data showed large and unexplained variations. The arterial CO_2 and pH both decreased, indicating a state of partially compensated acidosis. While Kamei (10) confirmed the occurrence of acidosis in rabbits and dogs, hyperventilation was not a constant feature, being present only when the concentration of CO in the inspired air was high.

The influence of CO on the circulatory system has received slight attention. Miura (11) found that in rabbits the cardiac output increased and the arterio-venous O_2 differences decreased with increased CO saturation of blood. In man, Asmussen and Chiodi (1) found only a slight increase of the cardiac output with CO poisoning ranging up to 33 per cent.

METHOD. The procedure followed was similar to that previously de-

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scribed (1) except that instead of breathing a $CO-O_2$ mixture in a closed circuit of small volume, a mixture of 0.15 to 0.35 per cent CO in air was inspired from a 600-liter Tissot. Since poisoning by this method was more gradual, we were able to obtain successive observations on ventilation, cardiac output and CO of venous blood. The details are as follows:

The subject, in the basal state, lay on the bed for approximately one hour before determinations were begun. Basal O_2 consumption, ventilation, pulse rate and blood pressure were recorded and arterial blood for pH was obtained. A basal cardiac output was then determined by Grollman's method.

The subject then began inspiring the CO-air mixture, or air alone, from the large gasometer, it being necessary to interrupt this only while making the observations on cardiac output. The total time during which the mixture was inhaled was never less than 70 minutes. The subject remained connected to the Tissot while the foregoing observations were repeated from one to three times during this period.

The procedure for determining the effect of CO on the respiratory response to CO_2 was as follows:

Pulmonary ventilation was measured breathing air and air-CO_2 mixtures. The subject was then given CO from a closed circuit filled with a $CO-O_2$ mixture. After 10 minutes ventilation was again measured using air and then the air-CO_2 mixture. The usual observations were made and samples of blood were taken for CO determination immediately before and after the final period of breathing the CO_2 mixture.

Experiments were also performed on dogs trained for metabolic observations. In the morning, after a fast of 16 hours, the unanesthetized dog was placed on a table and fitted with a leather mask over his muzzle. Lanolin and a rubber bandage made the mask airtight. The flow of inspired air from outdoors and of expired air into a gasometer was regulated by means of light rubber valves. Heart rates were recorded continuously by a Guillemin cardiotachometer.[2] The cardiac output was determined utilizing the Fick principle. Mixed venous blood was obtained from the right heart and arterial from the femoral artery. The procedure followed before and during the CO poisoning was similar to that used in the human subjects.

The methods of analysis employed have been previously described (1). The details of the determination of pH are to be found in the paper by Dill et al. (5), with the difference that the corrections for unsaturation were made on the basis of available hemoglobin capacity rather than on total hemoglobin capacity. The total hemoglobin was determined by saturating the blood with CO and analyzing it in the Van Slyke apparatus. Total ventilation was reduced to 0°C., 760 mm. Hg and dryness for calculating

[2] Manufactured by Victor Guillemin, Jr., 68 Jason St., Arlington, Mass.

the metabolism, and to 37°C., prevailing barometric pressure and complete saturation for estimating the minute ventilation.

RESULTS ON DOGS. The data on two Irish terrier bitches each weighing approximately 9.5 kgm. are shown in table 1. Lady had the same lung ventilation during CO poisoning as in the averaged control experiments, even though the saturation exceeded 50 per cent. In her the plasma pH showed a slight acid shift. In Dee the pH change was more pronounced. However, the lowest pH, 7.13, was found following a period of vomiting induced by the poisoning.

TABLE 1

Respiratory and circulatory effects of CO poisoning in dogs

DOG	EXPERIMENT NO.	VENTILATION	O_2 USED	A-V O_2 DIFF.	CARDIAC OUTPUT	PULSE RATE	pH_s	BLOOD LACTATE	TOTAL Hb CAPACITY	HbCO	REMARKS
		l. per min.	*cc. per min.*	*cc.*	*l. per min.*			*mgm. %*	*vol. %*	*%*	
Dee ♀ 9.5 kgm.	1–7		74	46	1.6	90	7.33		19.6	0	Average of 7 experiments
	8		61	37	1.6	145	7.28	10	21.4	20	
	9		67	37	1.8				21.7	34	
	10		67	35	1.9	130			19.6	35	
	11		62	32	1.9	136			17.8	38	
	12		76	29	2.6	179	7.25		20.6	41	
	13		68	22	3.0				20.5	43	
	14		82	20	4.1	204		50	21.9	50	
	15		94	35	2.7	210	7.13	21	22.2	53	Vomited
Lady ♀ 9.7 kgm.	1–4	1.2	68	45	1.5	93	7.32	17	20.5	0	Average of 4 experiments
	5	1.4	66	45	1.4	106	7.30		22.3	25	
	6	1.2	62	40	1.5	130	7.29	7	23.2	37	
	7	1.6	74	30	2.4	150	7.33	15	21.1	48	
	8	1.3	66	26	2.4	148	7.30	10	23.0	52	

There was a decrease in the average arterio-venous O_2 differences with higher CO. Although the cardiac output seemed to increase progressively, there was a rather abrupt rise when the hemoglobin saturation with CO exceeded 35 to 40 per cent. In three experiments on Dee with CO saturations of 35 to 38 per cent, the cardiac output ranged from 1.8 to 1.9 liters, but jumped to 2.6 liters when the HbCO was 41 per cent.

The blood lactates were within the normal range whenever determined, with only one exception. The heart rate tended to increase with a greater saturation of hemoglobin with CO.

RESULTS ON MEN. The four subjects were F. C., 28 years, 173 cm., 88 kgm.; S. H., 29 years, 162 cm., 74 kgm.; H. C., 33 years, 170 cm., 77 kgm.;

B. C., colored, 21 years, 170 cm., 64.5 kgm. The first three were subjects for one set of experiments, and the third and fourth for the other. When subjected to CO, the ventilation of H. C. decreased slightly (see tables 2 and 3). In F. C. no consistent differences were observed, and S. H. showed no respiratory response. The alkali reserve (T_{40}), measured on

TABLE 2

Arterial blood during CO poisoning in man

SUBJECT	EXPERIMENT NO.	VENTILATION	T_{40}*	pCO_2	$(BHCO_3)_s$	pH_s	TOTAL Hb CAPACITY	HbCO	O_2 ART. SATURATION	REMARKS
		l. per min.	*vol. %*	*mm. Hg*	*vol. %*		*vol. %*	*vol. %*	%	
H. C.	1		46.0	43.7	51.6	7.33	18.9	0	96.2	
			45.8	47.5	51.4	7.29	19.1	6.3	97.3	Recovery†
	2	7.2	47.5	41.9	54.1	7.37	19.3	0	97.7	
		6.9	47.8	40.0	55.2	7.39	20.6	7.2	90.7	Recovery
	3	7.0	46.3	46.3	52.4	7.31	19.4	0	97.1	
		6.5	46.7	45.1	53.2	7.33	19.9	4.6	98.3	0.15% CO in air‡
	4	6.9	47.6	42.8	54.2	7.36	19.6	0	96.6	
		6.7	47.4	45.6	54.1	7.33	20.0	9.8	97.4	0.3% CO in air
	5	6.6	48.1	39.3	55.1	7.40	19.1	0	95.7	
		6.9	47.4	43.7	54.0	7.35	19.8	9.2	95.2	0.3% CO in air
F. C.	1	8.2	47.2	43.8	53.8	7.34	19.8	0	95.7	
		7.5	47.0	48.9	53.6	7.29	20.8	10.4	97.0	0.3% CO in air
	2	6.6	48.9	40.0	55.9	7.40	19.2	0	96.1	
		6.9	47.6	43.9	54.2	7.35	19.5	9.5	98.5	0.3% CO in air
	3	6.7	46.4	43.8	52.8	7.35	20.0	0	96.3	
		6.7	46.6	44.9	53.1	7.33	19.9	3.4	97.7	0.2% CO in air

* Total CO_2 content of the arterial blood when pCO_2 is 40 mm.Hg.

† Blood drawn 10 minutes after the end of the rebreathing period.

‡ Blood drawn while the subject was breathing the mixture.

several occasions in H. C. and F. C., was within the usual range preceding and during the poisoning. This implies that no lactic acid or other fixed acid accumulated. Blood lactates, determined in most cases, were always low. The pCO_2 was elevated when the subjects had high CO concentrations. In all such cases the shift in pH was toward the acid side: this clearly depends on a depressed respiratory function. In two experiments

with moderate CO poisoning, the pCO_2 showed a slight decrease and the pH a slight increase.

TABLE 3

Respiratory, metabolic and circulatory effects of acute CO poisoning in man

SUBJECT	EXPERIMENT NO.	VENTILATION	O_2 USED	A-V O_2 DIFF.	CARDIAC OUTPUT	PULSE RATE	BLOOD PRESSURE	HbCO	REMARKS
		l. per min.	*cc. per min.*	*cc.*	*l. per min.*		*mm.*	%	
H. C.	1		221	56	3.9	61		0	
			218	42	5.1	76		41	Recovery
	2		221	56	3.9	58	100/70	0	
			246	42	5.8	87	120/80	35	Recovery
			246	46	5.3	78		32	Recovery
	3	6.6	239	55	4.3	62		0	
		6.9	240	40	5.9	98		42	
	4	7.1	240	55	4.3	68		0	
		6.8	261	43	5.9	103		52	
	5	7.1	240	62	3.8	64	96/72	0	
		6.8	242	59	4.1	70	102/66	16	
		6.9	238	40	5.8	81	104/72	32	
		6.8	242	43	5.6	92	106/64	45	
	6	5.9	230	61	3.7	62		0	
			230	59	3.8	62		0	
			230	60	3.8	62		0	
	7	6.0	225	55	4.0	61	124/84	0	
		5.9	219	53	4.0	70	116/78	23	
		5.8	231	45	5.1	80	118/78	43	
	8	5.9	230	55	4.1	65	108/76	0	
		5.9	220	53	4.0	72	104/72	25	
			228	50	4.5	76	110/74	30	
		5.4	228	45	4.9	82	107/68	39	
	9	6.1	235	62	3.7	64	102/74	0	
		6.0	226	50	4.5	76	108/74	31	
	10		228	55	4.0	66	106/74	0	
			218	42	5.1	79	106/72	33	
	11		221	53	4.1	67	104/72	0	
			221	54	4.0			0	
			223	38	5.7	76	108/74	32	

TABLE 3—*Concluded*

SUBJECT	EXPERIMENT NO.	VENTILATION	O_2 USED	A-V O_2 DIFF.	CARDIAC OUTPUT	PULSE RATE	BLOOD PRESSURE	HbCO	REMARKS
		l. per min.	*cc. per min.*	*cc.*	*l. per min.*		*mm.*	%	
F. C.	1	6.6	277	46	6.2	69		0	
		6.9	287	36	7.9	82		48	
	2	7.5	277	57	4.7	66		0	
		7.3	276	40	6.7	70		35	
	3	7.1	273	53	5.1	65	120/82	0	
		7.1	267	46	5.7	74	112/78	28	
				45	5.9		114/82	34	
		7.4	269	44	6.0	86	132/92	43	
	4	6.7	269	53	5.0	66	114/80	0	
		6.7	269	50	5.3	73	120/90	17	
		7.2	274	57	4.6	68	114/90	22	
		6.9	268	58	4.5	78	120/90	27	
	5	6.7	267	48	5.4	62	114/88	0	
		6.4	266	51	5.1	62	116/84	0	
		6.4	267	46	5.7	82	116/80	41	
	6	6.0	270	55	4.8	63	114/80	0	
		5.9		51	5.1	63	116/82	0	
		6.2	266	51	5.2	63	118/86	0	
		5.8	263	54	4.8	62	118/86	0	
S. H.	1		252	50	4.9	61	112/88	0	
			257	52	4.8	62	102/72	0	
			252	58	4.2	60	104/74	0	
	2		234	57	4.0	59	120/89	0	
			233	55	4.2	68	114/84	22	
			238	49	4.7	69	126/80	32	
			250	46	5.3	73	114/82	41	
	3		226	57	3.9	56	110/80	0	
			229	56	4.0	62	110/80	0	
			235	56	4.1	60		0	
	4	6.0	225	54	4.1	61	120/94	0	
		6.3	229	52	4.3	64	126/90	23	
		6.1	227			72	124/92	34	
		6.4	229	50	4.5	76	120/92	42	
	5	5.8	224	50	4.4	60	110/86	0	
		5.7	217	52	4.1	62	116/86	24	
		6.3	252	49	5.0	74	122/84	35	
		5.7	229	40	5.6	84	116/78	44	

In three subjects the average arterio-venous O_2 differences remained within the usual limits of day-to-day variations. Control determinations were made before each experiment. Three or more control determinations of the arterio-venous O_2 differences done during one day showed only slight changes. The A-V O_2 differences in the three subjects showed a

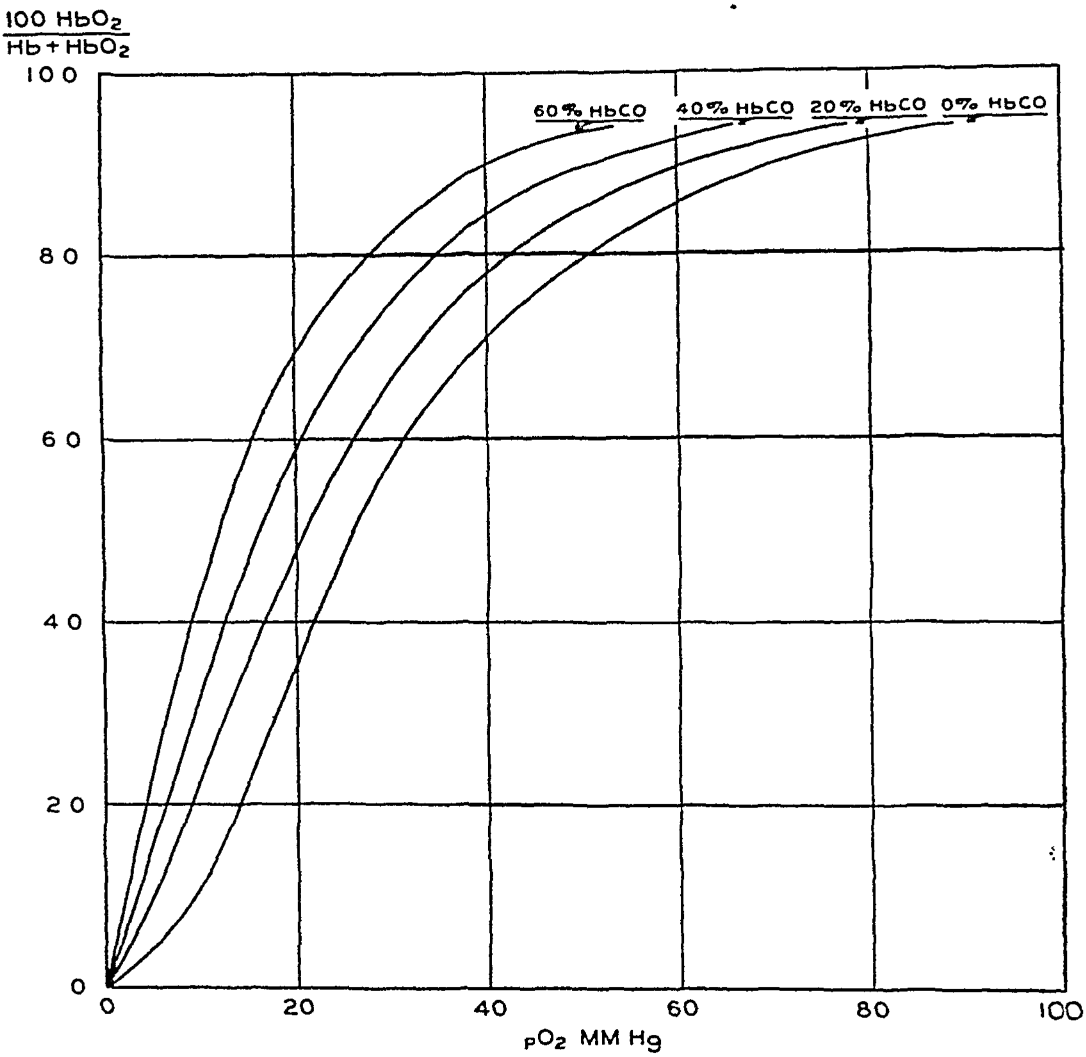

Fig. 1. Oxygen dissociation curves calculated according to Stadie and Martin in the presence of different percentages of HbCO.

moderate, progressive decrease when the concentration of CO in the blood was over 30 per cent. Therefore, the cardiac output increased, since the O_2 consumption remained unchanged. The increase in cardiac output was over 20 per cent, but not higher than 50 per cent, when the HbCO reached values of 40 per cent or over. With HbCO concentrations below 30 per cent, the changes in output were negligible. The pulse rate was increased when great amounts of HbCO were in the blood. The blood pressure also showed some elevation, although this was not a consistent finding.

Four oxygen dissociation curves, corresponding to 0, 20, 40 and 60 per cent HbCO, have been calculated according to Stadie and Martin (15) and are shown in figure 1. The values for the constants in the Stadie-Martin equation that most nearly accord with our dissociation curve for normal human blood are $N = -2.26$ and $\log K_{O_2} = -3.21$. Using these figures, the average venous pO_2 can be estimated with greater facility, as the following example will show (table 5). When the hemoglobin is 20 per cent saturated with CO, the available hemoglobin is 15.6 volumes per cent instead of 19.5. If the oxygen utilization is 5.3 volumes per cent, subtraction of this value from 15.6, and extending the value so obtained (10.3) to the corresponding oxygen dissociation curve, the venous pO_2 is found to be 27 mm.

The effect of CO hypoxemia on the excitability of the respiratory center was studied in two subjects (table 4). Concentrations of CO_2 in the inspired air varied from 1.8 to 5.2 per cent. The increase in ventilation as brought about by CO_2 is shown in terms of differences from the basal determination (2nd column). The third column shows the differences in ventilation obtained after the subject had been poisoned with CO. The two values for per cent of HbCO were obtained before and after the second period of CO_2 breathing. In each subject poisoned with CO, CO_2 produced less hyperventilation than in control experiments.

Discussion. Our observations and those of Asmussen and Chiodi (1) on man do not confirm the results of Haggard and Henderson (6) that dogs respond differently to CO while being poisoned than during recovery while breathing air. In contradiction to the findings of other investigators (6, 7) in both man and dog, no hyperpnea was evident, even though the concentration of HbCO in blood reached 52 per cent. Our results are in agreement with the clinical findings of Sayers et al. (13). Perfusion of the carotid body by Comroe and Schmidt (3) showed that as long as the arterial pO_2 remained sufficiently high no hyperpnea occurred despite reduction of oxygen content by CO. There is no reason for supposing that the arterial pO_2 is much reduced by CO poisoning. The elevation in arterial pCO_2 implies a slight reduction in alveolar pO_2, but this difference, perhaps 5 mm., would not reduce the arterial pO_2 below 75 or 80 mm. Hg. Since an increased pCO_2 coupled with a decreased pH ordinarily stimulates the respiratory center, the failure to observe hyperpnea in our experiments can only lead to the conclusion that the center was depressed. The failure of a given concentration of CO_2 in inspired air to induce as great an increase in ventilation in the hypoxia of CO poisoning as in the normal state is additional proof of depression. Dill and Zamchek (4) have shown that in a hypoxemia induced by breathing low oxygen, CO_2 has the opposite effect, i.e., an augmentation of the response to CO_2. These effects indicate that the chemoreceptors are essential in man to a potentiation of a hyperpnea when the arterial pO_2 is low.

There is much experimental evidence (14) that when the chemoreceptors of the carotid and aortic bodies of dogs are inactivated, hypoxemia depresses the respiratory center. So it appears that in man the depressant

TABLE 4

CO_2 hyperventilation in CO poisoning

SUBJECT	EXPERIMENT NO.	CO_2 PERCENTAGE	Δ_1*	Δ_2†	HbCO‡
			l. per min.	*l. per min.*	%
H. C.	1	1.79	2.2	1.5	30–26
	2	2.70	3.5	3.2	41–38
	3	3.35	5.4	4.1	33–29
	4	2.79	3.8	2.7	32–27
	5	4.87–5.09	10.7	10.1	34–26
	6	3.21	5.0	4.5	16–13
B. C.	1	3.18	4.4	4.3	0
	2	3.08	3.4	3.8	13–11
	3	3.10	4.0	3.0	33–29
	4	3.08	4.1	3.3	31–27
	5	5.22–5.03	13.5	12.2	32–24

* Δ_1 = difference between normal ventilation and hyperventilation induced by CO_2.

† Δ_2 = difference between ventilation breathing air and hyperventilation induced by CO_2 when subject is poisoned.

‡ Measured immediately before and after breathing CO_2-air mixtures.

TABLE 5

Venous pO_2 as estimated from O_2 dissociation curves of figure 1

	MAN AT REST WITHOUT CO	AT REST WITH 20% HbCO	AT REST WITH 40% HbCO	MAN IN MODERATE WORK WITHOUT CO	IN MODERATE WORK WITH 20% CO
Arterial HbO_2, vols. %	19.0	15.2	11.4	19.0	15.2
O_2 transport, vols. %	5.3	5.3	4.5	9.9*	9.3*
Venous HbO_2, vols. %	13.7	9.9	6.9	9.1	5.9
Venous saturation $\frac{100\ HbO_2}{Hb + HbO_2}$	68.5	62.0	57.5	45.5	36.9
Venous pO_2, mm. Hg.	37.0	27.0	20.0	24.0	17.5

* As determined by Asmussen and Chiodi (1).

effect of CO on ventilation is similar to that produced by ordinary hypoxia in animals with denervated carotid and aortic bodies. The carotid and aortic bodies have an arterial flow so abundant that their pO_2 is essentially arterial. Since they depend for their stimulation upon the pO_2, they are not stimulated in the hypoxia of CO poisoning.

In agreement with most investigators, except Haggard and Henderson (6), a shift toward an acid pH was found. The alkali reserve (T_{40}) was not appreciably altered. This is to be expected in view of the unchanged blood lactate and the brief periods of hypoxia, which lasted slightly over an hour. The lack of a hyperpnea with an acid pH makes it difficult to believe that there is a simple relation between pH and ventilation.

Circulation. The arterio-venous O_2 differences decreased and the cardiac output increased after the CO concentration exceeded 30 per cent. The failure of Asmussen and Chiodi (1) to observe any significant increases during CO poisoning can be explained by their use of lower concentrations, ranging from 23 to 33 per cent. These effects of CO poisoning on the cardiac output are comparable to observations made during the anemias. Nielsen (12) followed the cardiac output during treatment of a patient suffering from pernicious anemia. The increase in arterio-venous O_2 difference occurred concomitantly with the increase in hemoglobin. Similar observations were made by Blalock and Harrison (2) on both acute and chronic types of anemia produced in dogs. An outgrowth of these and other experiments was the hypothesis that the capillary or tissue O_2 pressure might be an important factor in the regulation of the output of the heart. Our present experiments tend to confirm this hypothesis. The rôles played by the increased pulse rate and by the vasomotor reaction in giving an increased cardiac output still remain to be elucidated.

While the hypoxemia of CO poisoning affects respiration and circulation much like simple anemia, its internal action is more severe because of the greater reduction in the pO_2 within the tissues. Stadie and Martin (15) have shown that, as a result of the shift of the oxygen dissociation curve to the left during CO poisoning, there is a diminished partial pressure of O_2 in the tissues when compared to conditions in anemia.

Our measurements of the arterio-venous oxygen difference and of the cardiac output have enabled us to estimate the magnitude of this effect in various stages of CO poisoning. The calculated curves (fig. 1) illustrate this clearly.

SUMMARY

1. No hyperpnea was observable during rest in either dogs or men when subjected to acute and severe CO poisoning. The CO_2 combining capacity was unchanged, the arterial pCO_2 was increased, and accordingly the pH was shifted toward the acid side.

2. In severe CO poisoning the respiratory center was depressed.

3. The cardiac output showed no more than slight increases with HbCO saturations ranging up to 30 per cent. From that level up to 50 per cent HbCO the cardiac output increased as much as one-half.

4. The direct action on the respiratory center of the acute hypoxemia

produced by CO poisoning that is severe yet compatible with life is purely depressive in nature.

5. From the data given the oxygen tension in venous blood can be calculated for various levels of HbCO.

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THE ACTION OF TEMPERATURE ON THE EXCITABILITY, SPIKE HEIGHT AND CONFIGURATION, AND THE REFRACTORY PERIOD OBSERVED IN THE RESPONSES OF SINGLE MEDULLATED NERVE FIBERS

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Physiologists long have felt that in the modification of physiological activity resulting from temperature changes they possess a valuable means of acquiring information relative to the problem of tissue functioning and it is a tool that has frequently been employed to this end. In the case of medullated nerve fibers the interpretation of certain of the effects of temperature is complicated by the congregation in nerve trunks of fibers of different characteristics. To obviate this complication we have, for some years, been studying the responses of *single* axons and now are presenting observations we have made on the effects of temperature on aspects of nerve response that require axon spikes for their interpretation or that can be studied advantageously by means of single axon spikes.

METHODS IN GENERAL. The nerve used has been the branch of the peroneal supplying the medial aspect of digit IV of *Rana pipiens*. Preparations were discarded that did not contain a fiber of outstandingly high excitability, and observations were confined to the responses given by that fiber.

Stimuli were condenser discharges initiated by a gas discharge tube activated through a delay circuit by the sweep of the cathode ray tube. The condensers available ranged between 0.1 and 0.5μF. Shock strength was controlled potentiometrically by a Leeds-Northrup Kohlrausch slide wire with 100 scale divisions readable to 0.1 division.

Three methods for altering the temperature have been employed. In some of the experiments the changes involved the moist chamber and all of its contents. In other experiments the temperature of the nerve was altered locally where it was in contact with one or the other of the pair of stimulating electrodes. These were calomel half-cells so designed (Blair, 1938) that Ringer's solution could be circulated through them. The solution entered the upper orifice of the electrode (fig. 1) and issued via the horizontal, terminal, slit-like orifice which is crossed vertically by the

nerve. The rate of flow of the solution could be adjusted so as to give the half-cell the desired temperature. The flow was rather rapid and the temperature of the fluid was taken immediately before its entry into the electrode. When the temperature of the solution differed widely from that of the chamber the temperature at the orifice of the electrode may have differed by a fraction of a degree from the recorded temperature. The outflowing solution drained back away from the nerve along the lower edge of the electrode, after coming into contact with about 2 mm.

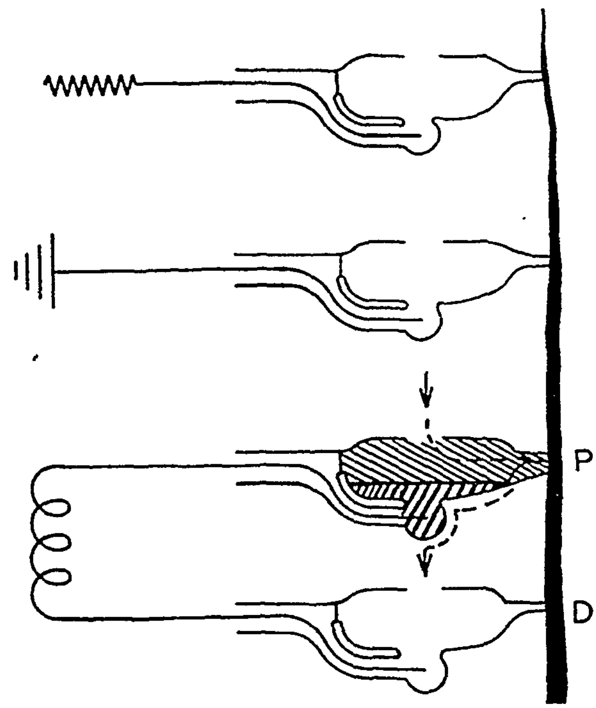

Fig. 1. Illustrating the arrangement of electrodes on nerve and method of changing temperature of nerve locally, in this case at the stimulated locus, *P*. The cross-hatching of electrode *P* indicates the contents of all electrodes: the lower level contains mercury and an emulsion of calomel and mercury; the upper level consists of Ringer solution. The solution that determines the local temperature takes the course indicated by the arrows and dotted line.

of the nerve's length immediately below the electrode. The flow produced not the slightest disturbance in contact, at least none that could be recognized when the electrode was the ground lead into the amplifier and amplification was maximal (about 2 mm. per μV). In this, the second of the cooling methods, the chamber was at room temperature while the nerve in contact with the half-cell was cooled. In the third method the chamber temperature was held constant at the low level while the temperature of the half-cell was raised to that of the room, when desired, by a continuous flow of the solution. The temperature of the chamber was taken at the level of, and close to, the nerve.

THE EFFECT OF TEMPERATURE ON EXCITABILITY. The electrical excitability of nerve as affected by temperature is a much studied subject. The earlier observers, using induction shocks as stimuli, concluded that cooling lowers excitability. Then it was reported by Gotch and Macdonald (1896) that when precautions are taken to circumvent the resistance changes of nerve caused by the temperature changes the result obtained upon cooling depends upon the "kind" of current employed: induction shocks disclosed a lowering of excitability, whereas constant current pulses longer than 5 msec. in duration, and condenser discharges, disclosed a raising of excitability. However, it was soon shown by Waller (1899) and by Lapicque (1907) that these differences are referable to the duration of the electrical currents employed as stimuli; currents of shorter duration decrease in effectiveness as the nerve is cooled, whereas currents of longer duration increase in effectiveness.

Method. Under the conditions of our experiments, the shocks employed by us for stimulation attain maximum height in less than 0.1 msec., and fall to one-third height in about 0.60 msec. (see fig. 2). The latter value

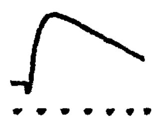

Fig. 2. Record (reproduced in photographed size) showing the configuration of the stimulating current. Time in 0.1 msec.

roughly approximates that of the spike at room temperature. If propagation and transmission of nerve impulses are by an electrical process the results obtained through the use of such a shock in temperature experiments should resemble somewhat those elicited by action potentials.

Results. Tested with these shocks, cooling a nerve invariably has raised its threshold. This result has not in our experience been altered by varying the resistance in the stimulating circuit, whether this was accomplished by adding resistance in series (up to as much as 1 megohm) or by varying the length of nerve subtended by the stimulating electrode through alteration of the position of the anode.

It can be shown, moreover, that the variation in the amount of current necessary to stimulate is the result primarily of the effect of temperature on excitability, rather than on resistance. Thus cooling only the region of the anode is without any appreciable effect on the threshold when, under similar conditions, cooling the cathode region produces the effect that is elicited through cooling the entire nerve.

The results of one of these experiments, carried out under the arrangements shown in figure 1, are collected in table 1. There it can be seen that upon cooling the nerve stretch that is in contact with the proximal

electrode, *P*, the threshold is raised when that electrode is the cathode, and not altered materially when that electrode is the anode. The results obtained when the distal electrode, *D*, is cooled are always very much less striking and they are variable. This variability is referable, we believe, to the relation the cooled stretch at *D* bears to the stretch of nerve polarized (*P*-*D*), the latter being mainly central to the former. However this may be, the results derived with *P* as the conditioned electrode are conclusive in themselves: any resistance change produced by the temperature change must be the same whether electrode *P* is cathode or anode.

TABLE 1

Effect of cathode or anode temperature on thresholds

RELATIVE THRESHOLDS	TEMPERATURE		CATHODE PROXIMAL (P) OR DISTAL (D)	CHANGE IN THRESHOLD $\frac{\text{WARM} - \text{COLD}}{\text{WARM}} \times 100$
	At cathode	At anode		
scale divisions	°C.	°C.		
592	22	22	D	
582	10	22	D	−1.7
567	22	22	D	+2.6
586	22	22	P	
575	22	10	P	−1.9
575	22	22	P	0
608	22	22	P	
710	10	22	P	+16.8
655	22	22	P	+9.2
705	10	22	P	+9.2
657	22	22	P	+8.8
622	22	22	D	
636	22	10	D	−0.9
636	22	22	D	0

Since the threshold changes only when it is cathode the change in threshold must be due primarily to the effect of the temperature on excitability.

In other experiments the temperature of the entire nerve was altered and determinations were made of the amount of current needed to stimulate with different interelectrode distances. Usually three electrodes were arranged on the nerve for stimulation. The electrode proximal with respect to the leads was cathode and in successive trials one or the other of the distal electrodes was made anode. In a crude way this procedure simulates that used by Rushton (1934) and by Cole and Hodgkin (1939) in determining separately the membrane and protoplasm resistance of nerve. They found that the slope of the resistance-versus-separation

curve is large for small electrode separations, but becomes smaller and finally constant as the separation increases; that "at 8 mm. the relation becomes constant with a gradient equal to the parallel resistance of the core and of the external fluid."

Under the conditions of our experiments the relation between electrode separation and the voltage necessary to stimulate becomes practically linear at about 5 mm. separation of the electrodes (see fig. 3). Of present

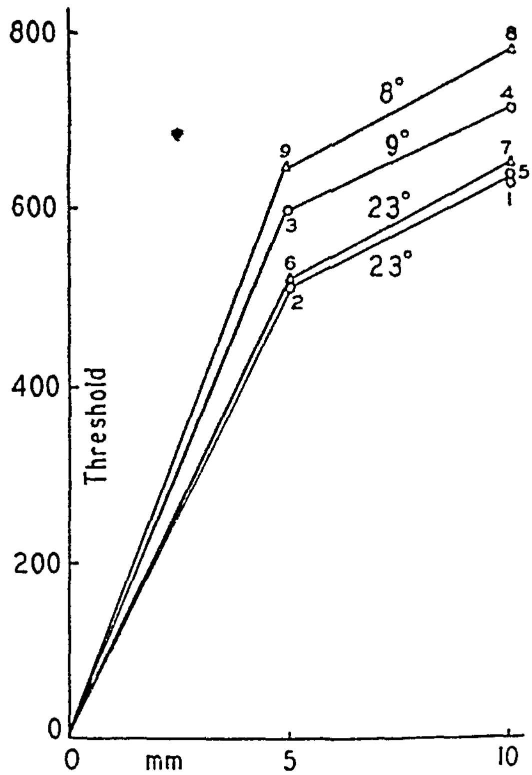

Fig. 3. Threshold *vs* interelectrode distance (resistance), the latter varied by altering position of anode of stimulating circuit. The sequence of the determinations and the temperatures are as indicated.

At the greater interelectrode distances the slopes of the curves are the same at the different temperatures.

interest, however, is the observation that beyond that separation, i.e., beyond 5 mm., the slopes of the curves are the same for different temperatures within the range tried, namely, 8° to 23°C. This result signifies that temperature alters excitability practically entirely through action on the membrane, or at least through action on the excited locus, and not perceptibly on the longitudinal resistance or on inactive nerve.

The effect of temperature on spike height and shape. In a study of the effect of temperature, Gasser (1931) has employed as the "axon spike potential" the multifiber record obtained through stimulating the sciatic

nerve of the frog with shocks submaximal for the A elevation while leading about 4 mm. from the stimulating cathode. Though under these circumstances many axon spikes contribute to the record, he found no temporal dispersion even when conduction involved the entire length of the nerve provided threshold fibers alone were stimulated. To "eliminate the diphasic artifact" and associated difficulties he killed the nerve "as close as possible to the active lead" and "then the observations were made promptly before deterioration of the end of the nerve or partial reformation of the new plasma membrane. . .". Under these circumstances he found that cold prolonged proportionally both the ascent and the descent of the spike and decreased its amplitude.

Method. We have met in another way the conditions specified by Gasser. In our final experiments we have used only those preparations the most excitable fiber of which transmitted impulses the entire interlead stretch, that is to say, only those yielding an action potential which initially was *completely* diphasic. Then the record was made monophasic by circulating isotonic KCl through the electrode acting as the distal lead, and the effect determined of changing the temperature of the nerve under the proximal lead, the rest of the nerve remaining at the temperature of the chamber (room temperature).

Failure to take these precautions accounts, we believe, for some variability encountered in the earlier experiments (to be referred to below). If initially the record happens to be monophasic, or nearly so, because of some fortuitous injury between leads, it tends with the lapse of time to become somewhat, and often unrecognizably, diphasic. Moreover, if this injured locus between leads happened to lie outside the range of action of the KCl applied through the distal electrode, the application of the KCl would not eliminate that diphasicity, and it might then go wholly unrecognized.

In determining the effect of temperature on the height and configuration of the spike of a single fiber it is essential that the recording mechanism be sufficiently quick to record the spike accurately. To test our mechanism a rectangular current was passed through the digital nerve via two calomel half-cells acting as electrodes. The lead into the amplifier was via two other calomel half-cells placed, about 6 mm. apart, on the nerve between those through which the current was applied. The recorded deflection attained 95 per cent of maximum in 0.16 msec. Since the fastest spike recorded in this investigation has had a time to maximum of 0.27 msec., and since the time to maximum at the higher temperature usually has been longer than 0.4 msec. and at the lower temperatures often as long as 0.8 msec., it is certain that the spikes have not been measurably distorted in the recording.

Results. Employing the precautions described above, cold invariably

has *prolonged the descent* of the spike *more than the ascent* and has *increased the height* of the spike; consequently it has increased enormously the area of the spike.

A typical experiment may be described. It is illustrated in figure 4, and the data are included in table 2 (expt. 5/23). Stimulation is indicated by the shock artifact. The interlead distance was 1.25 cm., which happens in this case to be the conducting distance, also. A and B are pictures obtained when the recording was frankly diphasic, A while the whole nerve was at the uniform temperature of 26°C., B while the nerve in the immediate vicinity of the proximal lead electrode was cooled to 8°C. The

TABLE 2

Effect of temperature on spike configuration and height

EXPERIMENT	INTERLEAD DISTANCE	CONDUCTION RATE AT GIVEN TEMPERATURE	TEMPERATURE	SPIKE ASCENT		SPIKE DESCENT		HEIGHT (RELATIVE)
				Duration	Ratio	Duration	Ratio	
	mm.	*M/sec.*	*°C.*					
5/15	11		25.1	0.35		0.50		100
			9.0	0.58	1.66	1.70	3.40	116
			24.5	0.39	1.49	0.52	3.27	100
	4		24.9	0.38		0.56		100
			9.0	0.58	1.53	1.06	1.89	110
			25.0	0.43	1.35	0.61	1.79	102
5/22	16		8	0.68–1.15		3.57–4.9		107–121
		20	25	0.52	1.3–2.21	0.755	4.73–6.49	100
5/23	12.5		8	0.55±		2.05		128
		26	26	0.39±	1.41±	0.50±	4.1±	100
5/24	16		8	0.62±		3.85		133
		27.6	26	0.37±	1.68±	0.51	7.54	100

presence of a shock artifact introduces some uncertainty into the location of the starts of the spikes. Accepting as the starts points selected somewhat arbitrarily, and allowing for some latency of response, the conduction time is found to be less than 0.50 msec. and the conduction rate, therefore, faster than 25 m.p.s., these with the entire nerve at 26°C. The interlead time, therefore, was slightly less than 0.5 msec. Since the time to maximum of the "warm" spike is 0.32 msec., its crest must have recorded at the proximal lead before the spike arrived at the distal lead; its ascending phase consequently must have recorded without deformation. This conclusion is confirmed by the fact that the height of the spike recorded at the proximal lead is not increased when, as will be explained below, it is pre-

vented from arriving at the distal lead (see fig. 4C). Then the temperature of the nerve at the proximal lead was lowered to 8°C. while the rest of the nerve remained at 26°C. The chief obvious result (B) is the prolongation of activity at the proximal lead; it now outlasts the process at the distal lead, so that the spike, as it affects the distal lead appears as a notch in the spike recorded at the proximal lead. Other minor changes referable to the temperature change are *a*, slight latening of the spike, and *b*, of the apex of the second phase, and *c*, broadening of the first elevation. The first two of these effects are manifestations of prolongation of conduction time,—they are slight because only a few millimeters, at the most, of the nerve's length are subjected to the temperature change.

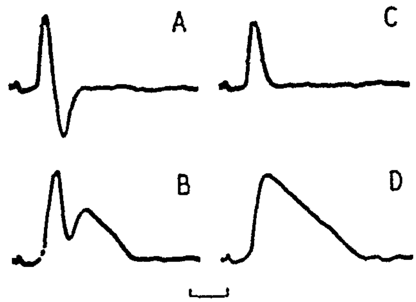

Fig. 4. Records (reproduced in photographed size) showing the effect of temperature on spike configuration (expt. 5/23, table 2).

A, completely diphasic; temperature of proximal lead 26°, of distal lead 26°. B, completely diphasic; temperature of proximal lead 8°, of distal lead 26°. C, completely monophasic; temperature of proximal lead 26°, of distal lead 26°. D, completely monophasic; temperature of proximal lead 8°, of distal lead 26°.

The small initial peak is the beginning of the shock artifact. Conduction distance = 1.25 cm. The bracket subtends 1 msec.

Having thus demonstrated that the fiber was conducting its full length, the recording now was made monophasic by the method described above, and records were obtained at the same two temperatures. They are reproduced as C and D. Except for the absence of the second phase these records match perfectly those of the first set: cooling prolongs the descent of the spike much more than the ascent and increases the height of the spike.

The data derived in this way from four preparations are included in table 2. Since temperature coefficients of nerve activities are dependent in a measure on the level of the subtended temperatures (Gasser, 1931), we refrain from presenting our data in terms of coefficients. It will suffice to say that in the range within which we have worked, the prolongation of the descent by cold always has been more than twice that of the ascent; in one case it was 4.5 times as great.

There are six other experiments, antedating those listed in the table, in

which initial complete diphasicity was not checked. In some of those experiments the stimulus was applied to the nerve at the proximal lead, in others at a remote point. In some the temperature of the entire chamber (of the entire preparation) was changed, in others the temperature at the proximal lead only. In some the nerve was killed by crushing, in others by the application of potassium chloride via the distal lead electrode. In all save one preparation cold increased the duration of the descent more than that of the ascent. In the case of the exception the ratio of the increase of the descent was 1.1, of the ascent 1.31; in other words, the ratios were alike within the limit of error. In view of the fact that there may have been a degree of concealed diphasicity in all of these earlier records we are not assigning any significance to this single exception to the rule.

Cold increased the height of the spike in all cases.

Cold is known to greatly enhance reflex activity of the spinal cord (see for example, Grundfest, 1941). Since cold increases spike height and duration these might be factors, along with others, such as the diminution of accommodation by cold, contributing to the increased activity of the cord.

THE RELATION OF THE ABSOLUTELY REFRACTORY PERIOD TO SPIKE DURATION AT DIFFERENT TEMPERATURES. *Method.* The ideal method for the determination of this relationship would be to compare the duration of the spike at the lead with the refractory period at thatl ocus. It requires, however, that there be at the lead only one fiber that will respond to a stimulus that is over 5 times as strong as the threshold of that fiber. Since this not a practicable condition, we have resorted to the method, previously employed by us (Blair and Erlanger, 1933), of stimulating one of the trunks of origin of the sciatic and recording in the phalangeal branch the spike of an adequate single fiber, when there happened to be one. The objection to the method lies in the fact, mentioned above, that the refractory period determined at one locus is compared with the spike duration determined at another.

Results. Gasser (1931), employing a multifiber response, found that "the temperature coefficient for the absolutely refractory phase is very close to that of the spike, but tends to be slightly larger, particularly in the lower temperature range."

Owing to the inadequacy of available methods, explained above, and since qualitatively we have obtained the same result as Gasser, we have carried through only two determinations. The results of one of these are plotted in figure 5. There it is seen that at the high temperature the discrepancy is narrow, that it is wide at the lower temperatures.

Here reference should be made to an observation, made in another place (Blair and Erlanger, 1933; also Erlanger in Erlanger and Gasser, 1937, p. 48), which casts some doubt on the value of these comparative determinations of the absolutely refractory period. In those determinations it

frequently happened, when dealing with fibers of low excitability, that the values for the absolutely refractory period were larger than those for the relatively refractory period. Such a state of affairs is, of course, wholly anomalous, and we consequently regarded it as an artifact produced by the very strong shocks needed under those circumstances. It still is possible, therefore, that the prevailing view (Adrian, 1914) is correct, that spike duration and absolutely refractory period actually are coterminate, though this remains to be demonstrated.

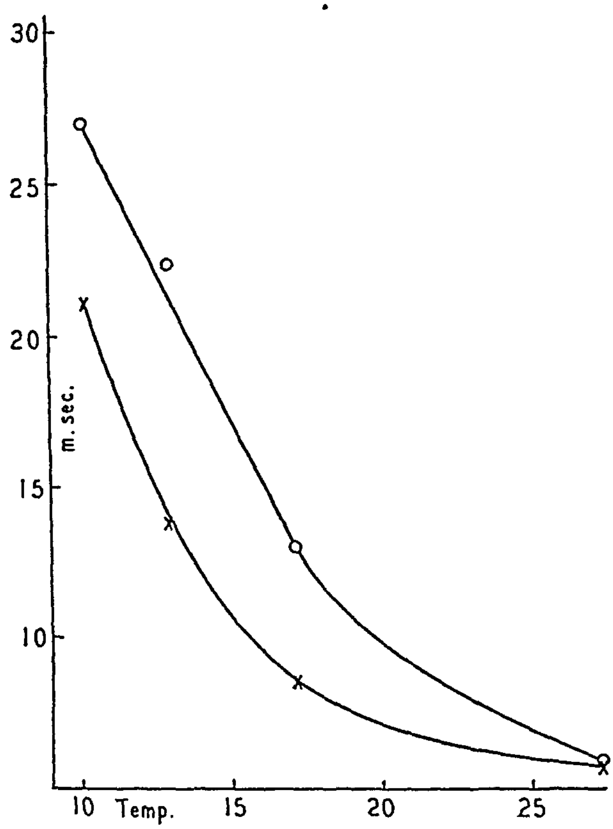

Fig. 5. Graph of a determination of the effect of temperature on the absolutely refractory period (circles) and spike duration (crosses).

SUMMARY

In observations made on the responses of single medullated fibers of the frog it is found that:

a. Cold increases the current strength required to stimulate, mainly through the effect of temperature on excitability, the effect on resistance being inappreciable,—

b. Prolongs both the ascent and the descent of the spike, but the descent *much* more than the ascent, and,—

c. Increases the height of the spike.

Reference is made to the possible relation of these effects to the enhancement of reflex activity by cold.

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A STUDY OF THE SPONTANEOUS OSCILLATIONS IN THE EXCITABILITY OF NERVE FIBERS, WITH SPECIAL REFERENCE TO THE ACTION OF STRYCHNINE

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When the most excitable fiber of a frog's nerve is stimulated with threshold shocks of uniform strength at relatively slow and regular rates not all the stimuli initiate conducted spikes. The range of shock strength between one that just fails ever to initiate any spikes and one that just suffices always to stimulate covered in our previous work about .2 per cent of the stimulating voltage. Associated with this "spontaneous" fluctuation in excitability there is a fluctuation in response latency. When two or more fibers happen to have about the same thresholds and are responding to the same threshold stimuli their excitabilities and latencies fluctuate quite independently one of the other, an observation which proves that the phenomenon is not referable to unrecognized fluctuations in the strength of the stimulus (Blair and Erlanger, 1933).

All of these observations have been confirmed by Pecher (1936; 1937; 1939). In addition, Pecher has concluded, on the basis of a statistical treatment of the subject, that the fluctuations in excitability are completely random, with the qualification that when the rate of stimulation is fast enough the exteriorization of the latency fluctuations may be modified by the resulting disbandment and facilitation (see Erlanger and Blair, 1940). Failing to obtain any clue as to their nature, Pecher concluded that the spontaneous variations are molecular in origin.

On the basis of this inference one would expect exposure of the nerve to conditions which alter molecular perturbability to change correspondingly the range of the spontaneous alterations in excitability and that conditions which change the nerve's excitability without altering molecular perturbability would be without influence on the spontaneous fluctuations. Of influences belonging in the first category temperature might be expected to have a definite effect; and in the second category one would expect to find local anode or cathode polarization and local treatment of the nerve with sodium citrate. This paper deals with the effects of these agents, and also of strychnine, on the amplitude of the spontaneous changes in excitability.

METHODS IN GENERAL. Observations have been made on the responses of single nerve fibers, employing the technique described in the preceding paper (Schoepfle and Erlanger, 1941). Again, the nerve used has been the branch of the peroneal supplying the medial aspect of digit IV of *Rana pipiens*; and all preparations were discarded that did not contain a fiber of outstandingly high excitability and the observations were confined to the responses given by that fiber. Usable preparations are obtained in about 30 per cent of the trials. The fineness of the digital nerve renders it particularly favorable for observations on the action of chemical agents, owing to the promptness with which their action becomes manifest (Erlanger and Blair, 1938).

Since the excitability of a fiber is constantly shifting in random fashion, though between certain limits, a single determination of the range is a time-consuming process, sometimes requiring several hundred stimulations (see below); and since under some of the imposed conditions it was difficult to hold the nerve in a constant state for any great length of time, determination of the parameters of strength-duration curves was out of the question. Consequently, for test stimuli we have resorted to shocks with a temporal configuration very roughly approximating that of a nerve action potential (see preceding paper).

The stimulation rate was constant during any set of observations, and usually about 1 per second. At the start of each set of determinations the sliding contact of the potential divider was set in such a position that the maximum sensitivity would be available that was compatible with the anticipated alterations in threshold, and then the resistance in the end coils was adjusted so that the selected position of the sliding contact was the threshold position. If, during the course of an experiment the amount of current necessary to stimulate happened to increase to the point where it exceeded the limits of the instrument thus set, all preceding data had to be discarded and the observations started again with another initial setting of the sliding contact.

Since the spontaneous changes in excitability are relatively small it was essential to have adequately constant stimuli. Determinations of the variability of the stimulating voltage were made at frequent intervals during the course of each experiment, employing a gas discharge tube activated by the stimulus discharging through a loud speaker as indicator. The full voltage varied less than 0.66 per cent and in some cases as little as 0.24 per cent. Since the *full* range of the spontaneous oscillations, when determined, has covered about 8 per cent of the threshold, it follows that variations of less than 8.3 per cent are, due to this source alone, within the range of error of the method $\left(\frac{0.66}{8} \times 100\right)$. As a matter of fact, we do not

regard as significant any induced change that is less than 20 per cent, unless the sign of the change is constant in successive experiments.[1]

It is not, however, practicable to employ as a measure the *full* range of the oscillations. The reason for this can be made clear by considering the configuration of the curve of the relation between the per cent of the stimuli that elicit responses and the strength of current in per cent of the absolute threshold, the absolute threshold being the current strength that just fails ever to stimulate. Such a curve is reproduced in figure 1. In this case the range of shock strength between one that elicits no responses and one that never fails to stimulate amounts roughly to 8 or 9 per cent of the threshold. Since the curve is S-shaped, the extremes of the range are

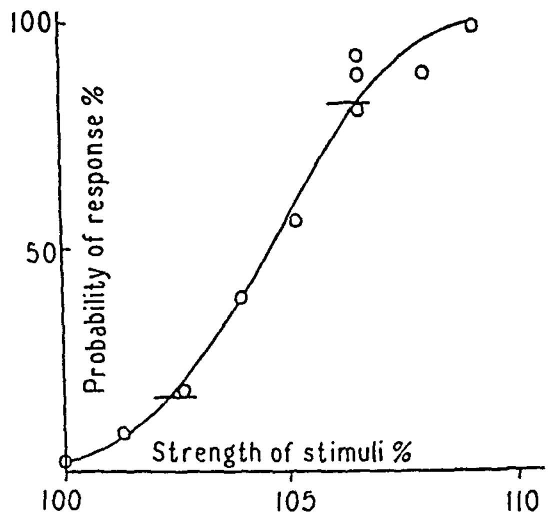

Fig. 1. Graph showing the relation between the probability of a response in per cent and the strength of the stimulus in per cent of the absolute threshold, assigning a value of 100 to the strength that just fails ever to stimulate. The horizontal lines subtend the part of the range measured, i.e., from 1 response to 6 shocks to 5 responses to 6 shocks.

difficult to determine experimentally. In the effort to minimize this difficulty we have adopted as end points the shock strength which elicits upon the one hand an average of one response in six stimulations, and upon the other five responses in six stimulations. Even so, the randomness of the variations necessitates long series of stimulations for each determination. In the present instance the range between one response in six stimu-

[1] Alterations in configuration and duration of currents also modify their effectiveness as stimuli. These have not been controlled. In view of the fact, however, that when two or more fibers of the same threshold are responding to threshold stimulation there is no correlation between their responses, it may be justifiable to assume that shape changes of the shocks have not qualified the results obtained.

lations and five responses in six stimulations amounts to slightly over 4 per cent of the threshold stimulation strength. This is a relatively high "normal" value, as may be seen in table 1 where the usual normals, as defined here and in the remainder of the paper, lie between about 2 and 4 per cent.

The relative amplitudes of the oscillations under the different conditions of an experiment are given in each case by the two limiting readings, but only when the nerve's resistance is not altered by those conditions. Any alteration of this resistance would cause an inversely proportional change in the value of the divisions of the potential divider. That is to say, if the resistance alone were to change, and not the oscillations in excitability, the "oscillation" ranges as read would be proportional to the "threshold" readings.

Though this assertion is an obvious requirement of Ohm's law when considered in relation to the fact that it is current and not voltage that stimulates, experimental verification, nevertheless, seemed desirable. This was accomplished by determining the effect of varying the interelectrode resistance by shifting the position of the anode of the stimulating current along the nerve. To cite the results of such a determination, increasing the interelectrode distance from 9 mm. to 27 mm. increased the average "threshold" readings given by the potential divider from 553 to 807, and the average of the oscillations from 18 to 27. The ratios of the oscillations to the "thresholds" are 0.0307 and 0.0299, that is to say, they are the same.

Since, then, the oscillation amplitudes as read will be proportional to any intercurrent resistance changes, and are proportional also to the real excitability range, the results of any imposed condition on oscillation amplitude require for their complete interpretation data on resistance. We have not, however, measured resistances and therefore must resort to recorded information, and this is scant, in discussing our results. It will be necessary to assume that all changes in oscillations as read on the potential divider actually are due to changes in excitability unless there are reasons for believing that the interposed conditions have altered in a known direction the nerve's resistance.

Anode and cathode polarization. *Methods.* The threshold of the nerve at the cathode of the stimulated locus has been raised or lowered by means of local anode or cathode polarization, respectively. This was accomplished by methods described elsewhere (Erlanger and Blair, 1936).

Results. Anode polarization (table 1, expts. 11/7 a.m., a and b, and p.m., 11/15) of an intensity that raises the threshold as much as 70 per cent has not altered the amplitude of the oscillation by an amount that significantly exceeds the probable experimental error, excepting one case (11/15) where the oscillations diminished 29 per cent. Moreover, the sign of the alteration has not been constant from experiment to experiment.

Cathode polarization (expts. 11/7 p.m., 11/15) that lowers the threshold

TABLE 1

Data on thresholds and oscillation amplitudes

EXPERIMENT	PROCEDURE	LOWER THRESHOLD	CHANGE OF THRESHOLD	OSCILLATION AMPLITUDE	OSCILLATION RANGE RELATIVE TO THRESHOLD	CHANGE IN OSCILLATION AMPLITUDE	REMARKS
		scale div.	*per cent*	*scale div.*	*per cent*	*per cent*	
11/7-a	Normal	590		22.0	3.7		
a.m.	An. pol.	838	+42.8	17.2		−21.8	
11/7-b	Normal	419		10.0	2.4		Same preparation, but another fiber
	An. pol.	728	+73.7	11.5		+10.5	
11/7	Normal	392		8.7	2.2		
p.m.	Cath. pol.	274	−30.1	9.0		+3.4	
11/15	Cath. pol.	435	−20.8	25.0		+19.0	
	An. pol.	637	+21.6	22.2		+5.7	
	Normal	524		21.0	4.0		
	An. pol.	749	+43.0	21.0		0	Polarization strength increased
	Cath. pol.	304	−42.0	13.0		−38.1	
	Cath. pol.	313	−40.3	16.5		−21.4	
	An. pol.	740	+41.2	14.8		−29.5	
11/25	Normal	743		25.0	3.4		
a.m.	0.7 NaCl	657	−10.4	22.3		−10.8	
	0.7 NaCl	605	−18.6	25.0		0	At end of about 30 min.
	Na citrate	250–170*	−66.2 −77.2	25–20−			About 40 min. treatment; at end the small fibers were repeating
p.m.	Normal	618		20.0	3.2		
	Na citrate	190–127	−69.2 −79.4	20.0±		0±	Terminated because fiber became inexcitable
11/28	Normal	918		25.0	2.7		
	Na citrate	515–450	−43.9 −51.0	28.7		+14.8	Readings started after 60 min. and continued for 44 min.
	Washed	600	−34.6	26.0		+4.0	
	Normal	875		22.5	2.6		Another part of nerve. New setting of potential divider
	Na citrate	342–310	−60.9 −64.6	41.6		+85.0	Readings started after 57 min. and continued for 60 min.
	Washed	610–700	−30.3 −20.0	29.6		+31.6	Readings begun after 25 min.

* Two values thus recorded in this column signify that the threshold changed through that range during the observation. This happened almost invariably during citration and occasionally during low temperature observations. Moreover, the return toward normal after citration is so slow that the "normal" thresholds at that stage always have been below the initial normal. When conditions could not be kept constant the order of the readings (high threshold-low threshold) was alternated throughout the period of observation.

TABLE 1—*Continued*

EXPERIMENT	PROCEDURE	LOWER THRESHOLD	CHANGE OF THRESHOLD	OSCILLATION AMPLITUDE	OSCILLATION RANGE RELATIVE TO THRESHOLD	CHANGE IN OSCILLATION AMPLITUDE	REMARKS
		scale div.	*per cent*	*scale div.*	*per cent*	*per cent*	
12/4	Normal	782		15.5	2.0		
	Citrate	350–200	−55.2 −74.4	16.4		+ 5.8	Readings begun after 40 min. and continued for 30 min.
	Washed	360–425	−54.0 −45.7	16.2		+4.5	After 22 min.
6/16	Normal	622		20.0	3.2		
	Citrate	215	−65.4 −59.8	20.3		+1.5	
	Washed	535		23.3		−9.1	After 30 min.
12/5 a.m.	23°	492		15.5	3.2		Temperature of entire chamber altered
	13.2–11.8°	905–942	+102 +91.4 +103	15–42.5		−3.2 +174 +12.8	Observations over a period of about 30 min.
	23°	444	+112	13.3		+219	
p.m.	23°	407		12.7	3.1		During and after the cooling period of this exp. there was obviously a progressive decline in oscillation amplitude. All comparisons are with the first room temperature readings
	12.8–11.0°	615–655	+51.1 +61.0	19.0		+49.6	Stim. rate 65/min.
	11.2–11.7°	620–645	+52.2 +58.5	15.0		+18.1	Stim. rate 30/min.
	11.7°	622	+52.8	13.0		+2.4	Stim. rate 65/min.
	21.0–22.5°	388–413		11.0			Stim. rate 65/min.
12/18	23.5°	437		15.2	3.5		Temperature of entire chamber altered
	13.4–10.0°	540–605	+23.6 +38.5 +24.2 +39.1	24.1		+58.5 +119	
	21.0–21.8°	435		11.0			
1/21	21.8°	593		17.2	2.9		Chamber maintained at room temperature; cathode temperature altered
	13.0°	754	+27.2	23.7		+37.8	

TABLE 1—*Concluded*

EXPERIMEN	PROCEDURE	LOWER THRESHOLD	CHANGE OF THRESHOLD	OSCILLATION AMPLITUDE	OSCILLATION RANGE RELATIVE TO THRESHOLD	CHANGE IN OSCILLATION AMPLITUDE	REMARKS
		scale div.	*per cent*	*scale div.*	*per cent*	*per cent*	
1/23	12.9°	801	+26.4	16.0		+1.9	Chamber maintained at low temperature; cathode temp. altered. Experiment proceeded at an unusually slow rate
	19.3–20.5°	605–663 (634)		15.7	2.5		
1/30	7.8°	504		21.0			Chamber maintained at low temperature; cathode temp. altered
	19.0°	366	+37.7	14.8	4.0	+41.9	
			+59.3			+41.9	
	7.8°	585		21.0			
4/28	Normal	572		15.7	2.7		
	Strych.	782	+36.7	55.0		+255	After 30 min.
	1:1000	753	+31.7	60.0		+282	After 60 min.
	Washed	430		15.0			After 60 min.
	Normal	492		15.0	3.1		
	Strych. 1:1000	650	+32.1	70.0		+367	
	Washed	527		22.5			New setting of potential divider
	Normal	528		21.7	4.1		
	Strych.	707	+33.9	90–55		+315	Two readings; time interval not mentioned
	1:1000					+153	
4/29	Normal	533		10.0	1.8		
	Strych.	644	+17.7	28.0		+180	After 60 min.
	1:100,000	648	+18.6	27.3		+173	After 105 min.
	Washed	730?		12.7			After 60 min.
5/4	Normal	528		11.8	2.2		
	Strych.	602	+14.0	58.3		+394	After 60 min.
	1:000,000		+12.3			+359	
	Washed	537		12.7			After 90 min.
6/27	Normal	600		10.0	1.7		
	Strych. 1:100,000	810	+35.0	15.0		+50	After 60 min.
	1:10,000	825	+37.5	20.0		+100	After 45 min.
				20.0+			
	Normal	580			1.7		After 60 min.

as much as 42 per cent likewise has failed to alter the oscillation amplitude by more than the experimental error, again with one exception (in the same experiment as the one mentioned above), in which the amplitude was diminished 38 per cent. And, as happened with anode polarization, the sign of the change produced by cathode polarization has not been constant.

Discussion. The cause of the variability of these results is not as yet clear. The possibility that uncontrolled demarcation currents may have been a factor is suggested by a comparable difficulty encountered in another connection (Blair and Erlanger, 1940), one that was eventually controlled by confining observations to portions of the sciatic nerve that were removed at least 10 mm. from any injured region. That span cannot be assuredly obtained in the digital nerve.

The increase and the decrease in the number of scale divisions needed to stimulate when nerve is polarized anodally or cathodally is usually ascribed to reduction or increase, respectively, in excitability. But Cole (personal communication) informs us that the steady state "resistance" of the squid membrane is increased at the anode and decreased at the cathode. It is possible, therefore, that our oscillation readings are too high while the nerve is anodally polarized, and too low while it is cathodally polarized. It is on account of this uncertainty that observations on the effects of polarization have not been multiplied. For the present we can conclude only that the observed changes in oscillation amplitude determined by polarization are within the limits of error of the method.

THE EFFECT OF SODIUM CHLORIDE. A single experiment (11/25), in which the nerve was treated with isotonic sodium chloride, gave negative results. The solution was applied by the method referred to below.

THE EFFECT OF SODIUM CITRATE. *Methods.* The solution used consisted of 90 cc. of 0.7 per cent sodium chloride and 10 cc. of 3.2 per cent sodium citrate. To apply it to the nerve, it was circulated slowly and steadily through the calomel half-cell acting as the cathode of the stimulating circuit (see preceding paper). It came into contact with the nerve in the immediate vicinity of the stimulating cathode only.

Results. In this concentration the citrate solution may cause the smaller fibers of the preparation to discharge spontaneously, but not the large fiber that is under observation. The threshold of this fiber may be lowered as much as 80 per cent by the treatment. The amplitude of the spontaneous oscillations is not changed (11/25 a.m. and p.m., 6/16) or is increased (11/28, 12/4). In one instance only (11/28) was the increase definitely greater than the experimental error.

Discussion. Though the citrate solution may increase the amplitude of the oscillations, the effect cannot be regarded as striking. What effect, if any, citrate exerts on the ohmic resistance of nerve does not seem to be known. The finding of Höber et al. (1939) that it does not alter resting potential may perhaps be taken to signify that it does not alter resistance.

On this basis one may be justified in concluding that if citrate solutions alter the oscillation amplitude at all, they increase it.

THE EFFECT OF TEMPERATURE. *Methods.* The temperature of the nerve has been lowered (or raised) by all of the methods described in the preceding paper.

Results. There are six sets of observations. In all cooling increased the oscillation amplitude. In five of the sets (12/50 a.m. and p.m., 12/18, 1/21, 1/30) the increase exceeded the limit of error; in one case (1/23) it was scarcely perceptible.

Discussion. Cooling the nerve increases quite decidedly the applied voltage required to stimulate. In the preceding paper evidence was presented indicating that this increase is due primarily to the lowering of *excitability* by cold, not to an increase in resistance. One must conclude, therefore, that the increase in the amplitude of the spontaneous oscillations elicited by cold is real.

Cold is known to increase reflex activity of the spinal cord (Grundfest, 1941). How an increase in oscillation amplitude might act to produce this effect will be considered under the discussion of the central action of strychnine. In the preceding paper it was found that cooling nerve increases the height of the spike and prolongs it, and it was there suggested that these changes might have the effect of increasing the efficacy of the spikes as stimuli. Both of these effects of cold, the effect on the spike and the effect on the oscillations, might therefore contribute to the reaction of the cord to cold.

THE EFFECT OF STRYCHNINE. *Methods.* Purified strychnine sulphate was dissolved in Ringer's solution free of HCO_3 and PO_4, and, thus dissolved, was applied to the stimulated locus by circulating it through the half-cell acting as the cathode. With one exception the concentrations employed have been 1:1,000,000, 1:100,000 and 1:1,000.

Results. The weakest of these solutions was without demonstrable effect. This result gives assurance that the modified Ringer's solution does not contribute to the results to be described.

Striking reactions were obtained with the other concentrations of strychnine. As may be seen in the table, the 1:100,000 solution (expt. 4/29, 5/4, 6/27) raises the threshold slightly, 14 to 35 per cent, but increases enormously the oscillation amplitude—as much as 394 per cent. This maximum is attained within one hour and is entirely reversible after one and one-half hours.

Similar effects were obtained with the 1:1,000 solution (expt. 4/28, *ter*). In this concentration the strychnine raises the threshold somewhat more, 32 to 37 per cent, but the order of magnitude of the increase in oscillation amplitude remains the same. In one case this solution annulled excitability irreversibly after 105 minutes.

The action of the strychnine solution on the spike requires mention here.

Treatment with the 1:100,000 solution long enough to bring out the effects described above has had no obvious effect on the amplitude or duration of the spike. There is but one observation with a 1:10,000 solution: the threshold was raised 37 per cent and the oscillations 100 per cent, at a time when there was no measurable effect on the spike. The 1:1,000 solution can produce its maximum effect on the oscillation amplitude at a time when the spike is altered only slightly; on the other hand, the oscillation amplitude may still be high at a time when the height of the spike is markedly reduced and its breadth increased (see fig. 2). The descent of the spike then is prolonged much more than its ascent.

Discussion. Changes in the configuration of the spike due to treatment with strychnine have not been alike in the experience of different investigators. Peugnet and Coppée (Coppée, 1936; Peugnet, 1936) state that it is modified by a 1:2,000,000 solution. The duration of treatment necessary to bring about this modification is not given, but they picture the effect of treatment with a 1:50,000 solution for 138 minutes and with a

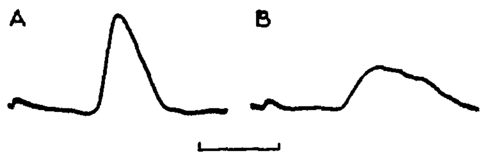

Fig. 2. Strychnine action on spike (expt. 4/28). A, normal. B, after 1:1,000 strychnine: ascent prolonged 33 per cent, descent 95 per cent; threshold raised 33 per cent; oscillations increased more than 300 per cent. Weaker solutions do not alter the spike.
The bracket subtends 1 msec.

1:10,000 solution for 20 minutes. The former modified the spike only slightly, the latter markedly. Heinbecker and Bartley (1939) state that the only effect of strychnine on the spike is to decrease its height. In our experience, as stated above, an obvious change in the spike has been obtained only with the 1:1,000 solution. Peugnet and Coppée found that the rheobase is lowered by concentrations of less than 1:1,000,000 and raised by greater concentrations. By our method of testing, strengths of 1:100,000 and 1:1,000 have raised the threshold.

Since, in our experiments, strychnine raised the threshold but slightly (not over 37 per cent), only a very small fraction, at the most, of the increase in the oscillation values can be referred to an effect of strychnine on nerve resistance. It would, however, be surprising if strychnine exerted any effect whatever on nerve resistance. There is every reason for believing, therefore, that practically the whole of the increase in oscillation amplitude produced by this agent is real.

The fact that different investigators fail to get the same change in the spike through exposure of the nerve to strychnine can be accounted for

only on the basis of unrecognized differences in technique or materials. The essential observation, from the present standpoint, is that the concentration, namely, 1:100,000, which in our hands has not obviously altered the spike, elicits oscillations that are as wide as any that have been noted with any concentration. And since the threshold is raised but little (it may be by only 12 per cent) at a time when the 1:100,000 solution is eliciting these wide oscillations, it seems quite possible (particularly in view of Peugnet and Coppée's observation that very dilute solutions *lower* the threshold) that there is a dilution at which oscillations still are at their maximum while the threshold is normal or lower than normal.

The striking increase in the amplitude of the spontaneous oscillations in excitability of nerve fibers that is elicited by strychnine suggests that this phenomenon may be a factor in the production of the central action of that alkaloid. Since, however, the oscillation swing has at no time carried the threshold, raised by the action of strychnine, below its normal level, it is obvious that the strychnine oscillations could not, without some collateral action, account for strychnine convulsions. However, one is reminded in this connection of the fact that strychnine convulsions ordinarily do not occur in the absence of a play of afferent impulses on the strychninized centers; that, moreover, the topical application of strychnine to both the dorsal and the ventral regions of the cord is requisite to the induction of tetanus (Dusser de Barenne, 1911). Is it not possible, therefore, that it is the lowering of the central threshold by afferent impulses that permits the spontaneous oscillations in excitability, increased in amplitude by the strychnine, to attain that threshold and so to produce the convulsions?

The electrical activity of the cord during strychnine tetanus, recently described by Bremer in a preliminary report (1941), prompts another hypothesis utilizing the effect produced by strychnine on the amplitude of the oscillation in excitability. "Everything suggests," Bremer writes, "that this neuronal synergy (of the cord) is effected by an intercellular action, truly electrical in nature, which spreads from an occasional focus with the speed of an explosion and synchronizes, over wide areas, the pulsations of autorhythmic elements possessing nearly identical individual frequencies." It may be that the autorhythm premised by Bremer is the rhythm of our spontaneous oscillations in excitability, increased in amplitude by the action of strychnine—that the rate of the "tetanic waves," 30–10 per second, is the rate of those oscillations. It is more likely, however, since this is the usual rate of discharge of anterior horn cells, that the "occasional focus" is a group of anterior horn cells, reflexly activated. The Bremer picture, then, would be reproduced by stimulation, through electrotonic spread from those cells, of neighboring cells which momentarily found themselves in the low threshold phase of their excitability oscillations, enhanced in amplitude by the action of the strychnine. This

stimulation would occur without synaptic delay, and so would be accounted for the "spread" of the process through the cord "with the speed of an explosion."

Heinbecker and Bartley (1939) have described other actions of strychnine, such as a diminution in accommodation and a prolongation of the period of latent addition, which, they believe, account for its central action. Such reactions to strychnine as these would, of course, tend to enhance any rôle the increase in amplitude of the oscillations in excitability might play in the induction of the central response to strychnine.

In so far as the foregoing considerations are valid they apply also to the enhancement of reflexes induced in the mammalian cord by cold, since while increasing the height and duration of the spike, cold increases the amplitude of the spontaneous oscillation; but cold raises the threshold more than does strychnine. Cold also diminishes accommodation.

Evidences of a Seasonal Variation in Amplitude of Oscillations. All of the determinations of the original series (Blair and Erlanger, 1933) were made during the second half of the month of May. As stated above, the average range of the oscillations in that series was about 2 per cent. In the case of the present series, the average of the first normal determinations of the preparations made during November to March, inclusive, is 3.2 per cent and of those made since then (beginning April 28th, but excluding the one of June 16th), 2.1 per cent. And several other determinations of the normal, made during June but not included in the table, have yielded percentages below 2. The determination of June 16th was made during a "cold" spell and gave a value of 3.2 per cent. This experience seems to indicate that "cold adaptation" increases the oscillation amplitude. The values for the *full* oscillations listed by Pecher (1939) range between 4 and 10 per cent and average 5.5 per cent; he gives neither temperatures nor dates.

General discussion. Three of the agents we have tested, namely, anode and cathode polarization and sodium citrate, are supposed to exert their effects on excitability through action on the "membrane". It is noteworthy that though all markedly affect electrical excitability, anode polarization lowering it and cathode polarization and citrate raising it, their action on oscillation amplitude in no case is striking if, indeed, they affect it at all. On the other hand, cold must affect all fiber mechanisms, and the fact that it enhances the oscillations while lowering excitability, considered in relation to the action of the above-mentioned agents, suggests that the oscillation response is not of the "membrane". The site of action of strychnine on the fiber still remains to be determined; but the fact that it lowers excitability while increasing the oscillations, may be taken to indicate that strychnine, also, produces the latter effect through action on something other than the hypothetical "membrane". But in view of its

nebulous state, the further development of this notion obviously is premature.

Previous work has disclosed evidences of subthreshold oscillations under certain imposed conditions. For instance, when frog's fibers are made to discharge repetitively through continuous superliminal cathode polarization there may be "gaps" in an otherwise regular series of responses. These gaps have durations which are about equal to two, or to three, or, rarely, even to four of the regular spike intervals, indicating that the gaps "result . . . from failures of an uninterrupted rhythmical oscillation in excitability to elicit a response" (Erlanger and Blair, 1936). And when the strength of a constant current is below the repetitive threshold, but above the rheobase, the make spike may be followed at the stimulated locus by a periodic variation in excitability decrementing through three recognizable oscillations (Erlanger and Blair, 1936). Monnier and Coppée (1939) have described a similar oscillation persisting after a brief subthreshold stimulus given to a nerve subjected to the influence of continuous superrheobasic cathodal polarization or of sodium citrate.

Arvanitaki (1939) has succeeded in recording local oscillatory variations in potential produced in giant fibers by cathode polarization of a locus treated with sodium citrate. And Brink and Bronk (1941) have produced similar oscillations in giant axons merely by removing the calcium and magnesium from topically applied sea water.

When these oscillations, however caused, develop under conditions that permit of the determination of their frequency it usually is found to be of the order of 200 to 300 per second. It remains to be determined whether the variations in excitability observed in the present investigation are periodic and if so what their rate may be. At the moment, therefore, it is not possible to assert that the oscillations in excitability and the rhythmical subconducted variations in potential are manifestations of one and the same phenomenon and therefore have the same frequency.

The faster rhythms exhibited by bits of frog's central nervous system may have a rate as high as 150 per second. This rhythm, and other central rhythms, have been attributed to "spontaneous activity of the cells" (Gerard and Young, 1937). The spontaneous oscillations of excitability we have studied are manifestations of nerve fibers. Is it possible that this fiber activity and this electrical activity of the central nervous system are fundamentally the same? If so we have here still another justification for regarding "axons as samples of nervous tissue" (Gasser, 1939).

The results we have obtained through the use of these agents fail to substantiate Pecher's inference that the oscillations are produced by molecular perturbations. Though cold would increase the mean free path of molecules, it is inconceivable that strychnine would have this effect.

SUMMARY

The amplitude of the spontaneous oscillations in excitability exhibited by single fibers in the phalangeal nerve of the frog is not changed by more than the error of the method, by

a. Anode polarization of a strength that raises the threshold as much as 70 per cent, or by

b. Cathode polarization that lowers the threshold 42 per cent, or by

c. Sodium citrate that lowers the threshold as much as 79 per cent.

d. The oscillation amplitude is definitely increased by cold which raises the threshold, but usually not more than 50 per cent.

e. The oscillation amplitude is increased as much as 394 per cent by a solution of strychnine which may not raise the threshold more than 12.3 per cent.

The possible relation of this effect of cold to the increase in excitability of the central nervous system that is produced by cold, and the possible relation of this action of strychnine on fibers to its central convulsant action are discussed.

The effect produced by strychnine on the oscillation amplitude renders untenable the view ascribing the oscillations to molecular perturbation (Pecher, 1939); and both the strychnine effects and the effect produced by cold suggest that the oscillations are not attributable to processes originating in the "membrane."

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THE EMPTYING TIME OF THE STOMACH OF OLD PEOPLE

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In a recent review Ivy (1) has noted the lack of quantitative information concerning important functions of the digestive system of the aged. Study of the normal physiologic functions of old people is basic to all phases of geriatrics. The present investigation was undertaken to determine what effect, if any, the aging process has on gastric emptying.

During the past few years the senior author and his colleagues have determined, under controlled conditions, the gastric emptying time of 59 vigorous, young men. These data have been of invaluable help in the present study, since they serve to establish a criterion for the emptying time of the stomach of young adults in their physical prime. The data obtained from the studies of gastric emptying of the aged group reported in this paper could be compared directly to those obtained from the young adults.

METHOD. Twelve men, the youngest of whom was 58 and the oldest 84, and whose average age was 70.8 years, were used for this study. Ten of the twelve subjects were indigents residing in the county infirmary; one was a college professor; one was a janitor.

At 7:30 a.m. they were given a high carbohydrate test meal similar to that used in previous studies (2). This meal consists of 15 grams of Quaker Farina and 350 cc. of water, boiled together and evaporated to 200 cc.; 50 grams of barium sulfate were added so that the position of the meal could be observed fluoroscopically. No food had been eaten since the preceding evening. The subjects were instructed to relax mentally and physically, but were allowed to walk around the laboratory if they so desired. The same methods were employed in all respects as for the estimations of gastric emptying time of young adults.

With the exception of 2 subjects, at least 3 determinations of gastric emptying time were made on each individual at exactly weekly intervals, to establish the mean for each subject. Time of emptying of the stomach as ascertained by fluoroscopic observation was determined to the nearest 10 minutes.

RESULTS. The results are expressed graphically in figure 1. The mean values for each of the aged subjects are superimposed upon a diagram of

distribution of the individual mean values for 59 young adults. The average length of time for the test meal to leave the stomach in the 12 old men was 1.94 hours, with extremes of 1.33 and 2.75 hours. The median value was 2.04 hours. The gastric emptying time of the 59 young adults averaged 2.08 hours, with extremes of 1.03 and 3.08 hours and a median value of 2.06 hours. It is apparent, without statistical analysis, that there is no significant difference between gastric emptying times of the 2 groups.

DISCUSSION. Recently it has been pointed out (3) that gastro-intestinal symptoms are common in old age although gastro-intestinal disease is relatively uncommon. None of the subjects used for the studies reported in this paper, as far as could be determined, suffered from organic disease of the gastroenteric tract or complained of gastric disturbances at the times

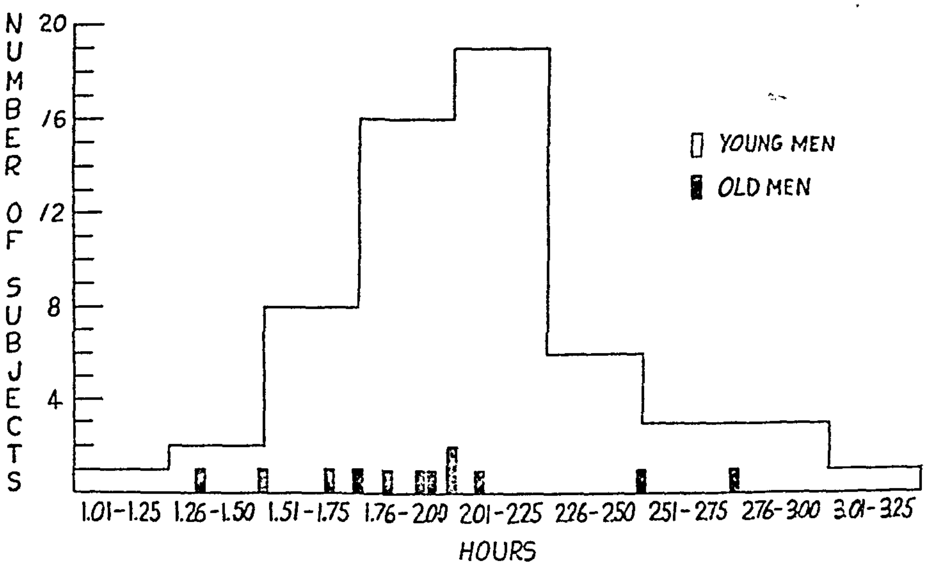

Fig. 1

the observations were made. They had normal appetite for food and regularly ate three meals each day. None suffered from cardiac embarrassment or other apparent causes of anoxic or stagnant anoxia, which could affect gastric emptying. All had sufficient cardiac reserve to perform light, physical tasks without distress. All but 2 coöperated well during the 4 weeks interval of study.

The subjects remained indoors during tests, since external environmental temperature may influence gastric emptying. The observations were made during December and January, while those on the 59 young adults were made during the cool months from October to May; thus was offered some degree of control of any possible seasonal variation in gastric emptying time.

Any greater activity of the younger group during the evening preceding the test meal, which is a factor difficult to control, presumably had little ef-

fect on mean values. If activity were greater, it should cause hunger, and Ivy and Fauley (5) have shown that hunger decreases gastric emptying time in experimental animals. The results indicate, however, that the younger group did not have a shorter gastric emptying time.

An interval of 7 days between tests is sufficient to rid the intestinal tract of all traces of barium sulfate. If much shorter periods were used, it is possible that a mass of barium sulfate in the lower intestinal tract could reflexly impede gastric emptying. Reflexes from the colon have been shown by Pearcy and Van Liere (6) to influence gastric motility.

Meyer and Necheles (3) have reported that no free acid was found in the fasting stomachs of 65 per cent of 29 patients over 60 years of age, and that salivary, gastric and pancreatic secretions were reduced in amount and content of enzymes, except for pancreatic amylase. An achlorhydric stomach may empty faster than the normal, but Ivy (1) has noted that this is not definitely proven. None of the 12 subjects was willing to submit to tests of gastric acidity, but even if it is granted that the majority were achlorhydric, the results still indicate that the gastric musculature of the aged is capable of exerting vigorous and effective peristalsis.

In the present small series, no correlation was observed between physical vigor and gastric emptying time. One of the least vigorous subjects had the most rapid emptying time.

Although there is no difference between gastric emptying times of old people and young adults, this should not be interpreted to mean that the aged should eat as much or even the same quality of food as is eaten by healthy, young adults. An assumption that gastric emptying time is a main criterion for eating habits is unwarranted, particularly in view of diminutions in the aged of secretions of the various digestive organs (3). Also, since many people past middle age lead sedentary lives, obesity results if eating habits are not suitably modified, and life expectancy may thereby be decreased.

SUMMARY AND CONCLUSIONS

The normal gastric emptying time of 12 men, the youngest of whom was 58, the oldest 84 and whose average age was 70.8 years was determined fluoroscopically. None of these subjects had demonstrable organic disease of the gastroenteric tract, and all were capable of performing light, physical tasks. The determinations of the gastric emptying time were made exactly at weekly intervals.

The criterion used for establishing normal gastric emptying time was the data secured from 59 vigorous young adults, who had been given the same type meal and subjected to exactly the same procedures as were the aged subjects.

The gastric emptying time of the 12 old men was 1.94 hours; the extremes

ranged from 1.33 to 2.75 hours. The gastric emptying time of the 59 young adults was 2.08 hours with extremes ranging from 1.03 to 3.08 hours.

It is concluded that gastric emptying is not influenced by senescence.

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OPTIMAL NaCl CONCENTRATION FOR ORAL SALINE DIURESIS[1]

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With the exception of the few workers who have ingested (0.9 per cent) isotonic NaCl and found it not to be diuretic, there have been few studies on oral NaCl solutions as diuretic fluids. Some physicians, not realizing that the sweat contains only 0.3 to 0.6 per cent of inorganic solids, have gone so far as to recommend 0.9 per cent NaCl solution as a drinking beverage for workers in hot atmospheres. Most clinicians have learned from edematous patients that one liter of 0.9 per cent NaCl supplies sufficient salt for the ordinary twenty-four hour requirements when no abnormal NaCl loss is occurring. It was thought that a careful study of the diuretic efficiency of a series of hypotonic concentrations of NaCl, as well as of the chlorides of calcium, magnesium, and potassium, might form an adequate basis for the future study of diuretics and at the same time furnish data on the all-important water and salt balance of the body. It is also known that while 0.9 per cent NaCl solution contains too much salt to be mobilized from the tissue spaces for diuretic purposes, at the opposite extreme the lack of salt in distilled water might inhibit diuresis when a larger dosage is used. Accordingly, the study of the diuretic effect of different concentrations within the range between these two extremes was first undertaken.

EXPERIMENTAL. All experiments were performed on female dogs which had been operated to produce bladder-extrophies (Dragstedt and Dragstedt, 1928). The animals were trained to stand quietly in stocks for the duration of each experiment, which lasted approximately three hours. Urine was collected at ten minute intervals from the funnels strapped under the fistulae. The urine volumes were measured and the chloride excretions were determined by a modified Volhard method (McLean and Selling, 1914). Water and food were withheld from the animals for five hours preceding each experiment to permit absorption of any recently ingested food or water. A control period of thirty minutes was allowed before the

[1] Aided in part by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

TABLE 1

Successive daily saline diuresis—100 cc./kgm.

		1st day	2nd day	3rd day
0.9% saline	Serum NaCl change	+71 mgm. %	+21 mgm. %	+66 mgm. %
	Urine peak	19 cc.	31 cc.	36 cc.
	Peak NaCl*	228 mgm.	337 mgm.	310 mgm.
0.5% saline	Serum NaCl change	−8 mgm. %	+22 mgm. %	+35 mgm. %
	Urine peak	43 cc.	55 cc.	54 cc.
	Peak NaCl	73 mgm.	81 mgm.	67 mgm.
0.2% saline	Serum NaCl change	+15 mgm. %	+15 mgm. %	+3 mgm. %
	Urine peak	50 cc.	57 cc.	56 cc.
	Peak NaCl	9 mgm.	42 mgm.	46 mgm.
Dist. H_2O	Serum NaCl change	−15 mgm. %	−15 mgm. %	−30 mgm. %
	Urine peak	40 cc.	45 cc.	48 cc.
	Peak NaCl	10 mgm.	15 mgm.	22 mgm.

* Expressed as mgm. NaCl/10 min./10 kgm. of dog. (See figs. 2 and 3.)

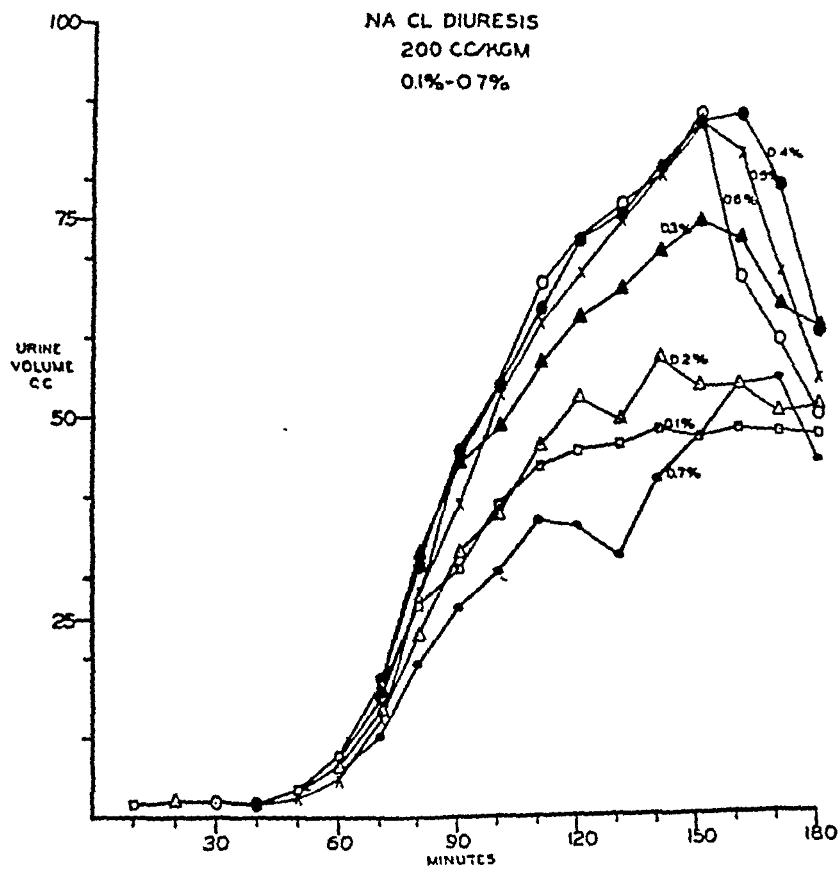

Fig. 1. The comparative maximum diuresis produced in the 10 kgm. dog by solutions of 0.1 per cent to 0.7 per cent NaCl when 200 cc./kgm. is given in 50 cc./kgm. doses at 30, 60, 90 and 120 minutes. The maximum diuresis occurs with doses of 0.4 per cent to 0.6 per cent, and this was found to obtain for doses of 100 to 300 cc./kgm. The diuretic response to the 0.7 per cent solution attains only the level produced by the more hypotonic solutions of 0.1 per cent and 0.2 per cent.

administration of any fluid. All fluids were administered by stomach tube at 37°C.

It was determined that a 50 cc./kgm. volume is well tolerated by the dog's stomach and that this quantity disappears from the stomach in

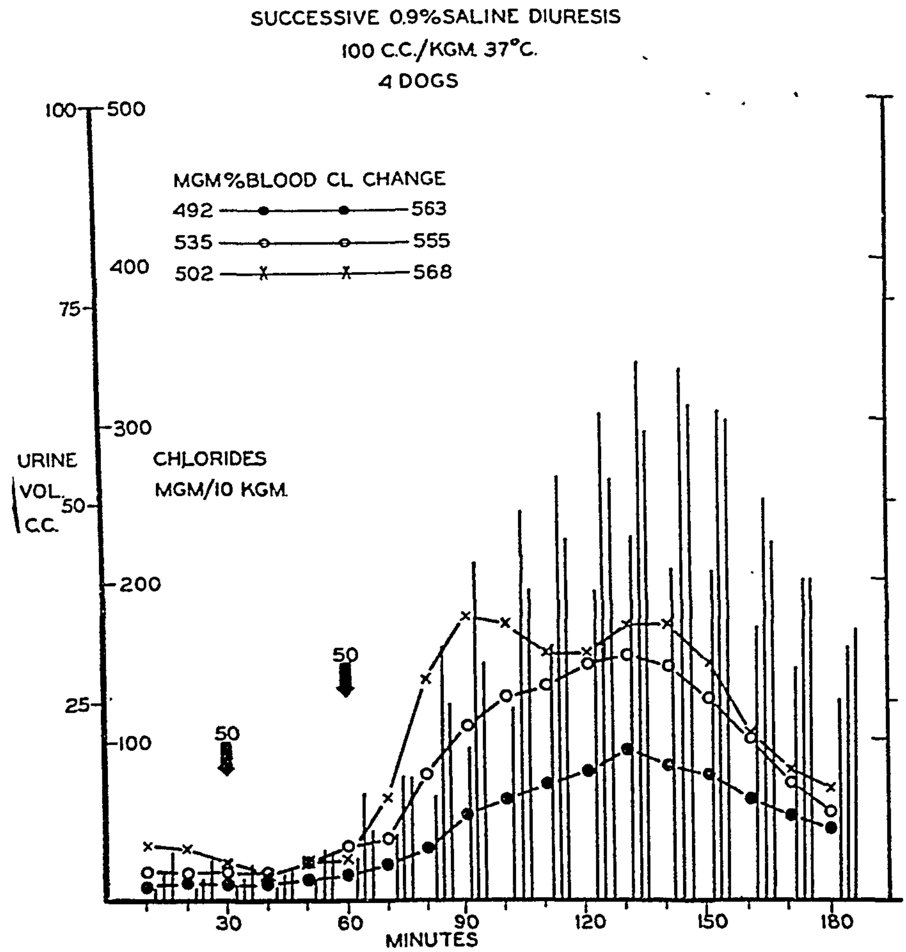

Fig. 2. Daily 0.9 per cent NaCl diuresis at 100 cc./kgm. The time of onset and peak of diuresis increased from day to day. The vertical lines on the inside of the ordinate measure the milligrams NaCl/10 min./10 kgm. The individual lines in each triad indicate respectively from left to right the height of chloride excretion at the corresponding time on the three successive days. The arrows indicate the time of administration of 50 cc./kgm. of the saline solution. The average blood chloride of the four dogs taken at the beginning and end of each experiment show a daily increase.

thirty minutes. Therefore, in these studies fluids were given in doses of 100 to 300 cc./kgm., divided into doses of 50 cc./kgm. administered at half-hour intervals. Four or five dogs were used in each experiment. Since the dogs used weighed approximately 10 kgm., all results were expressed on the basis of a theoretical 10 kgm. dog. The urine samples from all dogs in the group were pooled at each ten minute interval, and

a 5 cc. aliquot was taken for chloride estimation. Every thirty minutes the volume and chlorides of ten minute samples were determined for each individual dog to ascertain individual variation. Dogs which deviated from the average were replaced. A second series of experiments was performed to determine the effect of various concentrations of NaCl and also distilled water when administered three days in succession in doses of 100

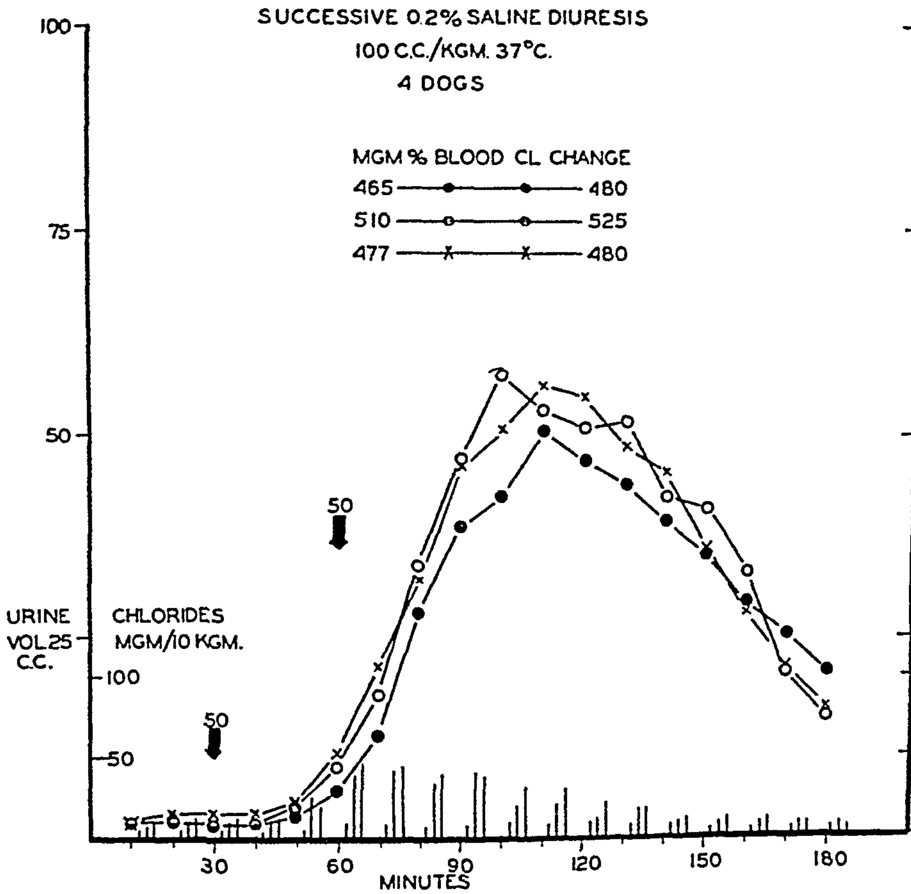

Fig. 3. Daily 0.2 per cent saline diuresis at 100 cc./kgm. More equal daily diuretic responses are obtained than with either 0.9 per cent or 0.5 per cent NaCl with respect to onset, peak and decline of diuresis. This may indicate that the disturbance in salt and water balance with this solution is minimum and easily compensated within 24 hours. No definite change in blood NaCl occurred.

cc./kgm. The blood chloride changes were followed daily with a sample obtained from each dog before the experiment and after the experimental diuresis. The samples were pooled and the chloride determination made in duplicate by the method of Koch. The summarized results are tabulated in table 1 and in figures 1 to 3.

Discussion. Oral administration of saline solutions ranging in concentration from distilled water to 0.9 per cent results in varying diuretic

responses, with the greatest diuresis occurring with solutions of 0.4 per cent to 0.6 per cent NaCl concentration. The limits of chloride excretion so far obtained in the normal 10 kgm. dog are 0.1 mgm./min. and 51 mgm./min. The difference between the diuretic response of the dog to 0.2 per cent and that to 0.5 per cent saline may represent the difference between sufficient chloride intake with 0.2 per cent saline and true chloride diuresis with 0.5 per cent NaCl. The oral administration of saline solutions for three days in succession results in the least average blood chloride change when 0.2 per cent NaCl is used. This concentration also furnishes adequate NaCl for the excretion of maximal quantities of fluid. Even with the extreme dilution of 0.1 and 0.2 per cent NaCl, a retention of over half of the NaCl occurred during the 3 hour diuretic period. The daily increase in urinary output is believed to indicate an increase in extracellular and tissue NaCl and, hence, an ability of the saturated tissues to absorb less NaCl and excrete more by way of the urine. This also indicates that even 0.5 per cent NaCl given orally is retained to such an extent that the dog is not in normal salt balance twenty-four hours later. Attempts were also made to substitute 0.5 per cent $NaHCO_3$ and 0.5 per cent NH_4Cl as a maximal diuretic fluid in order to study the effect of acid base balance on diuresis. These solutions caused marked vomiting and diarrhea and could not be given in doses greater than 50 cc./kgm.

In an attempt to verify these results in the human subject, three males ingested over a period of 90 minutes 50 cc./kgm. (4 liters) of 0.5 per cent NaCl solution. After an interval of one week the same amount of 0.25 per cent saline was ingested, and the next week distilled water was used. The 0.5 per cent saline experiment produced marked diarrhea in all three subjects. The 0.25 per cent saline was well tolerated and produced a diuresis almost comparable to the distilled water ingestion. The ingestion of 4 to 5 liters of distilled water over a period of one hour produced a headache in one subject and malaise in the other two subjects. Since the greatest diuresis accompanied the distilled water, it was thought that 50 cc./kgm. was not sufficient distilled water to exhaust the NaCl reserves of the body. The subjects noted a sustained diuretic effect from the 0.5 per cent saline, which carried over to the second and third day after the experiment.

With these results in mind, it is of interest to review the findings of Abel (1914), who in order to avoid "edema" in his vivi-diffusion experiments found it necessary to reduce the NaCl content to 0.55–0.6 per cent. Clark (1913) also found that the ordinary Ringer solution is improved if 0.3 to 0.4 per cent of the NaCl is omitted and the solution made isotonic with sucrose.

There are probably two underlying factors involved in the findings that 0.5 per cent NaCl is the most diuretic in dogs, while only 0.2 per cent is

needed for maintenance of salt balance. The absorption from the gastro-intestinal tract is more rapid when 0.5 per cent is used, but once this is absorbed the kidney cannot excrete more than 0.2 per cent NaCl in diuretic quantities. The vomiting which occurred with the lower concentrations of NaCl was probably due in part to the retention in the stomach of the dose of fluid previously administered.

SUMMARY

When bladder-extrophied dogs are given orally solutions of NaCl from 0.0 per cent to 0.9 per cent, it was found that 0.5 per cent NaCl produces the greatest diuresis; 0.2 per cent NaCl produces the least change in the NaCl balance, as shown by lack of cumulative action and minimal change in the serum chlorides. Human subjects cannot tolerate water containing more than 2 grams/liter of NaCl when large doses are given.

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THE DIURETIC EFFECT OF POTASSIUM, CALCIUM AND MAGNESIUM GIVEN ORALLY IN SALT SOLUTION

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It has been known since the pioneering experiments of Ringer (1882) on the perfusion of the frog heart that potassium, calcium, and magnesium each contribute to the automaticity of heart muscle. Aside from the clinical data which attest to the diuretic activity of these three ions in overdosage, little is known regarding their diuretic effect on the intact animal when used in a physiological range of concentration. Accordingly, diuretic experiments were planned in which these ions in physiological concentration would be added to the physiological, and most diuretic, concentration of NaCl.

TABLE 1

EXPERIMENT	DOGS	CC./KGM.	NaCl	K	Ca	Mg
			per cent	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
I	8	200	0.45			
II	8	200	0.45	18		
III	8	200	0.45		10	
IV	8	200	0.45			3.5
V	8	200	0.45	18	10	
VI	8	200	0.45	18	10	3.5

Since the serum level of NaCl, 0.45 per cent, was found to be the most diuretic in previous experiments, a solution of this concentration was used as an appropriate medium in which to administer the chlorides of potassium, calcium and magnesium. Four trained, female, bladder-extrophied dogs were used in these studies. Experiments were performed at weekly intervals to allow the animals to return to a normal salt and water balance. Two experiments were performed at each saline concentration, making a total of eight dogs for averaging purposes. Solutions were administered by stomach tube in such quantities that each animal received a total of 200 cc./kgm. This was divided into four doses which were given

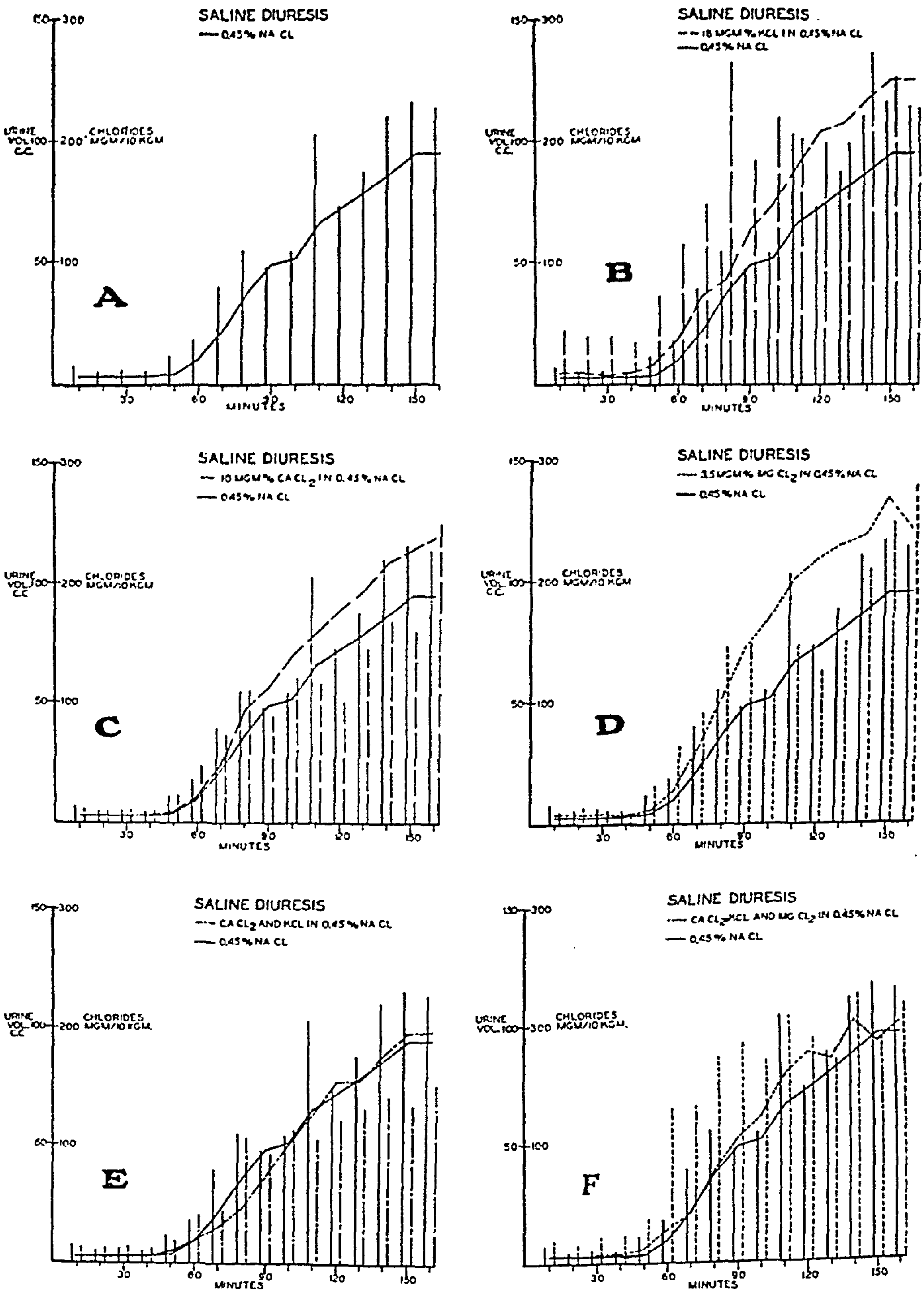

Fig. 1. Data are expressed on a basis of a 10 kgm. dog when eight dogs received 200 cc./kgm. orally in doses of 50 cc./kgm. at the 30, 60, 90 and 120 minute intervals. The milligrams chloride excretion, expressed as NaCl, is represented by the vertical lines.

at half-hour intervals after an initial thirty minute control period. Table 1 represents a protocol of the experiments conducted.

RESULTS. The graphs of figure 1 summarize the experimental data. After determining the normal response to 0.45 per cent NaCl, KCl was added in an amount to provide 18 mgm. per cent of available K ions in the diuretic fluid. For purposes of comparison this curve is superimposed on the control NaCl graph. The onset of diuresis is earlier and a higher peak is attained. The chloride excretion, as represented by the broken vertical lines, appreciably exceeds that of the 0.45 per cent NaCl alone.

When $CaCl_2$ was added (Ca^{++} = 10 mgm. per cent), the water diuresis exceeded that of the control curve. The chloride excretion in the 90 to 150 minute period was less than that of the control 0.45 per cent NaCl experiments.

$MgCl_2$, when added in sufficient quantity to provide 3.5 mgm. per cent of available Mg ions, resulted in a marked increase in water diuresis over the entire period of the experiment. The NaCl output, except for the 50 to 90 minute period, equaled that of the 0.45 per cent NaCl.

The combination of Ca (10 mgm. per cent) with K (18 mgm. per cent) resulted in a loss of the normal diuretic effect of these ions. A definite retention of chloride started at 90 minutes and obtained for the duration of the experiment.

The addition of Mg (3.5 mgm. per cent) in the presence of Ca (10 mgm. per cent) and K (18 mgm. per cent) decreased the diuretic effect of the Mg, Ca and K ions. A greater chloride excretion occurs with this combination than with the 0.45 per cent NaCl alone.

DISCUSSION. When K, Ca, and Mg chlorides are given singly in a diuretic fluid in the same concentrations in which they appear in the blood stream, the resulting diuresis is at all times greater than that seen with 0.45 per cent NaCl alone. In the case of a balanced solution containing Ca and K in 0.45 per cent NaCl, the data point to a retention of chloride ions and a total reversal or loss of the diuretic effect of these two ions. The diuretic effect of $MgCl_2$ is also decreased by administration in a balanced Ca-K-Na solution. The explanation of these phenomena must await further studies checking blood volume and tissue water balance. The lack of water diuresis might be accounted for by increased urinary concentration. The decrease in chloride excretion, however, would indicate an actual retention of these ions. This antagonism between Ca and K has been confirmed in water balance studies in rats.[1] These results are at variance with those observed with the heart-lung-kidney preparation (Eichholtz and Starling, 1925). When K or Ca chlorides were given

[1] To be published.

separately, no effects were noted in the excretion by the isolated kidney. If these two salts were given together, however, there was an increase in chloride excretion and water output. The concentrations used in those experiments, however, exceeded the normal physiological range.

SUMMARY

By adding each of the serum cations to an oral 0.45 per cent NaCl solution it is possible to ascertain the diuretic effect of each ion. The addition of Ca is the least diuretic, while Mg addition results in the greatest diuresis. K is intermediate. The combination of Ca and K prevents the diuretic action of each ion and results in a greater retention of chlorides. Mg does not completely enter into this antagonism, although the diuretic effect of this ion is greatly diminished.

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THE ENZYMATIC INACTIVATION OF CHOLECYSTOKININ BY BLOOD SERUM

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We have recently demonstrated (1) that blood serum contains "secretinase," the presumed action of which is to remove active secretin from the circulation and permit pancreatic secretion to subside. We believe this enzyme to be an important factor in the regulation of the external secretion of the pancreas.

In the normal digestive process the hormonal stimulus for pancreatic secretion is accompanied by an emptying of the gall bladder which is due to contraction of its musculature mediated by the hormone cholecystokinin (2). The characteristic gall-bladder contraction in response to cholecystokinin stimulation is abrupt and reaches its height in one or two minutes; this is followed by slow relaxation. The extent of contraction and the interval required for relaxation depend to a large extent on the quantity of cholecystokinin administered. When an extract of intestinal mucosa containing both secretin and cholecystokinin is injected and the responses of the pancreas and gall bladder are observed, the parallelism is striking with respect to onset, magnitude and duration of action of the two hormones. A similar parallelism obtains in the case of their endogenous absorption in the course of the normal physiologic response to a meal. In general on intravenous administration the cholecystokinin effect is the more protracted.

Since secretin and cholecystokinin enter the circulation from the same source (the upper intestinal mucosa) and appear to be closely related chemically, and since their separate effects coincide to some extent, a similar or identical agency in clearing them from the circulation appeared to us to be probable. The presence of such a mechanism was tested in the experiments described below.

EXPERIMENTAL. 1. *Materials.* The same S_1 preparation employed by us in our previous work served as the cholecystokinetic agent. This material, which contains one unit of secretin in 0.25 mgm., causes a 1-cm. rise in intra-gall-bladder pressure in most dogs in a 0.5 mgm. dose. In the present work injections of 2 mgm. were made in order to secure pronounced reac-

tions and accentuate any differentiations in effectiveness encountered in the course of treatment by serum. Normal dog serum was used throughout these studies.

2. *Methods.* Large dogs (15 kilos or over) were anesthetized with sodium pentobarbital and the abdomen was opened by a midline incision. The cystic duct was clamped and a trocar inserted in the dome of the gall bladder and connected to a Becker tambour to provide for a recording of gall bladder motility (2). Provision for recording pancreatic secretion and carotid blood pressure was made as in our previous experiments. The response of the animals to a control injection of 2 mgm. of S_1 was established, and mixtures of S_1 and serum were injected under the following conditions: (a) *Constant:* Volume, pH, and temperature of serum.

TABLE 1

Progressive disappearance of cholecystokinin from solutions of 2 mgm. S_1 in 10 cc. dog serum at varying incubation times (37°C.)

DOG NO.	GALL-BLADDER RESPONSE													
	Rise	Duration	Rise	Duration	Rise	Duration	Rise	Duration	Rise	Duration	Rise	Duration	Rise	Duration
	cm.	*min.*												
1	3.7	12	3.5	12	3.3	8	3.0	7	0	0			4.0	15
2			2.0	9	1.8	8	1.5	6	1.0	4			3.0	17
3					4.0	18	3.0	15	2.0	8			3.5	14
4	5.3	13	4.1	11			1.7	16	0.7	4	0	0	5.0	12
5	6.7	15	4.5	12	4.0	9	2.0	8	1.8	6	0	0	6.1	14
6	4.8	9	4.6	8	3.3	7	2.5	7	2.3	7	0	0	5.0	10
	Incubation time (hours)													
	0		½		1		2		3		5		Control	

Varied: Time of incubation. (b) *Constant:* Volume and pH of serum and time of incubation. *Varied:* Temperature of incubation. (c) *Constant:* Time and temperature of incubation and pH of serum. *Varied:* Volume of serum. (d) *Constant:* Time and temperature of incubation and volume of serum. *Varied:* pH of serum.

The degree of excursion of the Becker tambour was standardized with reference to the observed height of the visible column of bile, so that the records obtained could be recorded in terms of changes in intra-gall-bladder pressure in centimeters of bile.

Results. The gall-bladder response to the intravenous injection of S_1-serum mixtures was altered in a manner analogous to the diminution previously noted in the response of the pancreas to the serum-treated material. The degree of inactivation of cholecystokinin was found to vary directly with the length of time of incubation, and was usually complete in

5 hours when 2 mgm. of S_1 was treated with 10 cc. of serum. The degree and duration of gall-bladder response in this series of animals is listed in table 1, and an illustrative record of one experiment is reproduced in figure 1. The inactivation was found to proceed slowly at low temperatures, optimally at body temperature, and was entirely eliminated by heating the serum to over 60°C. prior to incubation (table 2). The degree of inactivation for a given time and temperature was a function of the amount of

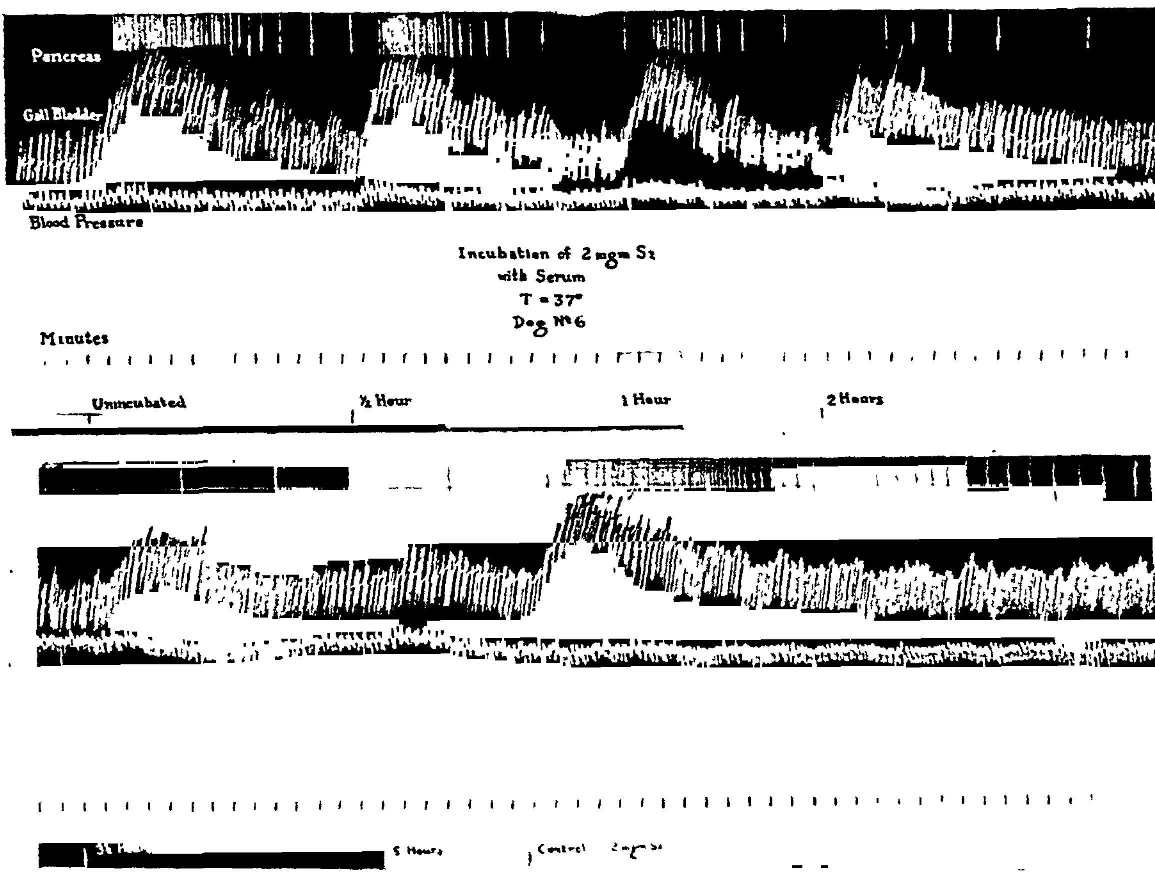

Fig. 1. Progressive inactivation of the secretin and cholecystokinin in 2 mgm. of S_1, following incubation with 10 cc. of normal dog serum for varying lengths of time. The secretin is entirely inactivated after 3½ hours, and the cholecystokinin after 5 hours.

serum used (table 3), and the inactivating agency was ineffective when the acidity or alkalinity was caused to deviate markedly from the normal pH of the blood (table 4).

It was not possible to evaluate alterations in gall-bladder response with the same degree of accuracy as in the case of the pancreas. The nature of the recording apparatus is such that only sizable differences in response are significant; and we were restricted to a small number of injections in any given animal, since it is known that frequently repeated stimulation of

the gall bladder by cholecystokinin will eventually throw the viscus into a state of persistent contracture which renders the animal useless for assay purposes.

TABLE 2

Effect of temperature of incubation upon gall-bladder response to 2 mgm. S_1 incubated with 10 cc. of serum

DOG NO.	GALL-BLADDER RESPONSE									
	Rise	Duration	Rise	Duration	Rise	Duration	Rise	Duration	Rise	Duration
	cm.	*min.*								
7	1.5	14	0.5	7	0.2	3			1.4	14
8	2.3	15	1.7	12	1.2	9			2.5	18
9	4.0	25	3.3	18	2.0	15			3.3	25
10	2.0	8	0.7	6	0	0				
11	1.0	6	0.8	6	0.5	4			1.0	6
12	1.2	9	0.9	9	0.6	4			1.2	9
13					0.5	3	2.5	18	2.2	18
14					0	0	1.8	15	1.2	13
	Temperature, degrees C.									
	10		22		37		60		Control	

TABLE 3

Effect of varying quantities of serum on gall-bladder response when incubated with 2 mgm. S_1 at 37°C. for 4 hours

DOG NO.	GALL-BLADDER RESPONSE											
	Rise	Duration	Rise	Duration	Rise	Duration	Rise	Duration	Rise	Duration	Rise	Duration
	cm.	*min.*										
15	2.2	13	2.5	9	1.5	6	0.5	2	0	0	2.5	11
16	2.5	14	1.7	8	0.8	5	0.6	4	0	0		
17	1.2	18			0.5	9	0.1	1	0	0	0.9	?
18	2.0	15			1.0	7	0.8	5	0.7	3	2.0	15
19	1.2	12	0.5	3			0	0			1.5	13
20	3.0	13	2.5	10	1.8	5	1.0	2	0	0	4.0	15
21	1.2	12	0.3	3	0	0	0	0			1.5	17
	Volume of serum (cc.)											
	1		2		5		10		20		Control	

Discussion. It is apparent from the data submitted that inactivation or destruction of cholecystokinin is brought about when this hormone is incubated with blood serum at the physiologic normal temperature and reaction. The process of removal of active cholecystokinin from such a

mixture is dependent on the time of incubation and the amount of serum used. It proceeds most rapidly at body temperature and is prevented by heating to 60°C. or by adjusting the reaction to an acidity or alkalinity deviating markedly from the normal (2 pH units or more). It follows that the mechanism whereby active cholecystokinin is caused to disappear from

TABLE 4

Effect on gall-bladder response to 2 mgm. S_1 incubated with 10 cc. of serum for 4 hours at normal, acid, and alkaline pH

DOG NO.	SERUM pH	GALL BLADDER	
		Rise	Duration
		cm.	*min.*
22	4.6	1.7	13
	7.0	0.2	3
	10.4	1.2	11
	Control	1.7	14
23	2.0	1.0	8
	7.0	0	0
	11.0	1.0	8
24	2.0	1.4	8
	7.0	0.7	5
	11.0	0.7	7
25	Control	2.2	12
	9.4	2.0	12
	7.5	1.2	6
	3.4	1.2	7
	Control	2.0	?
26	3.4	1.5	12
	7.5	0.3	3
	9.4	1.2	13
27	3.5	2.5	11
	7.5	1.0	5
	10.2	2.7	18
	Control	2.5	12

the incubation mixtures is an enzymic one, since the usual criteria for the demonstration of enzyme activity have been fulfilled by the experiments outlined above.

It remains to be established whether the same enzyme is involved in the inactivation of both secretin and cholecystokinin. It is quite conceivable that the two hormones are structurally similar chemically; witness their

close association *in vivo* and in processes for preparing concentrates. Thus it is entirely admissible that a single enzyme present in the blood destroys or alters a certain chemical group or atomic linkage essential for their separate activities. However, until the structures of the two substances are known, one can merely speculate regarding this possibility. It is certain that they are not identical; if additional proof of this fact were necessary, the present experiments supply it. Each injection of 2 mgm. of S_1 contained 8 units of secretin and 4 units of cholecystokinin; thus if a single substance were being destroyed by the blood serum, the cholecystokinetic effect would obviously have completely disappeared before the secretin potency had been lost. Exactly the reverse was actually obtained, as is illustrated in figure 1. It follows that the enzymic inactivation of secretin proceeds with considerably more rapidity than that of cholecystokinin.

SUMMARY AND CONCLUSIONS

A concentrate containing secretin and cholecystokinin has been incubated with normal dog serum and inactivation of the cholecystokinin has been demonstrated.

Such inactivation is progressive with time of incubation and is most rapid at 37°C. The agency involved is heat-labile and is effective only at a reaction approximating the pH of the blood. Thus the mechanism whereby inactivation occurs is an enzymic one.

The enzyme involved may or may not be secretinase. The inactivation of cholecystokinin proceeds considerably more slowly than does that of secretin.

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RED CELL COUNTS, PERCENTAGE VOLUME, AND THE OPACITY OF SUSPENSIONS[1]

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Several methods have been suggested as a means of avoiding direct red cell counting and hematocrit determinations, the former because it is tedious, and the latter because of the doubt which exists as to the right rate and time of spinning.[2] One method is described by Ponder, Dubin and Gordon (1934), and consists in finding the red cell count by dividing the percentage volume found by hematocrit by the mean cell volume in μ^3 found by diffraction. Another is that originally due to Oliver (1895–96) and developed by Holker (1921); the red cell count is determined from the opacity of a suspension, measured in various ways. Shohl (1940) has used the same principle for finding the percentage volume, the opacity of a suspension of red cells of the sample of blood being measured with the Evelyn photometer, and the percentage volume read off from a previously prepared linear calibration curve.

I re-examined the opacimeter method in 1934 (Ponder, 1934), and was unable to find the precision which Holker claimed for it, for the opacity of a red cell suspension turned out to be determined not only by the number of cells, but also by their size, their shape, and the refractive difference between them and the surrounding medium, as well as by other factors which are obscure. In general, the discrepancy between the results obtained by opacimetry and by direct counting were found to be sometimes as great as ± 10 per cent.

In these experiments, however, I used a Zeiss Stufenphotometer with a large angular aperture, and so both scattered light and directly transmitted light were measured together. The importance of the angular aperture has since been emphasized by Mestre (1935), and so better results might be obtained with a photometer of smaller aperture such as the Klett-

[1] This investigation was carried out by the aid of a grant from the Simon Baruch Foundation.

[2] For a description of the difficulties which arise in connection with the hematocrit method, see Ponder (1940) and the papers therein referred to. It should be again emphasised, however, that the value of the method depends on whether it is used for the determination of relative, or of absolute, volumes.

Summerson photometer.[3] I also used a filter with a maximum transmission at 5300 A, but Shohl has since pointed out, on the basis of Drabkin and Singer's investigations (1939), that a red filter with a maximum transmission at 6600 A ought to be better, as it minimizes the effect of variations in hemoglobin content. Lastly, my investigation was directed more to seeking the sources of error of the method than to seeing how it would work out in practice, which is the purpose of the present series of determinations.

METHODS. The samples of venous blood (about 5 ml.) were received into small bottles containing heparin. Oxalate should not be used, because of its effect on the cell volume and on osmotic properties (Ponder, 1940). The cases from which the blood samples were taken were selected so as to exclude the blood dyscrasias, and those showing low red cell counts were cases of either secondary anemia or hemorrhage.

The opacity measurements were made with a Klett-Summerson photometer, using *a*, the red filter 66 with a transmission of from 6400 to 7000 A, and *b*, the green filter 53 with a transmission of from 5000 to 5700 A. The suspension was prepared by adding 20 $mm.^3$ of the heparinized blood sample to 10 ml. of 1 per cent NaCl or to 10 ml. of the citrate-formol solution described by Shohl (3.0 per cent sodium citrate to which is added 1 ml. of formalin for each liter). Readings of the photometer were made as soon as possible after adding the blood to the NaCl or citrate-formol and mixing, 30 seconds after mixing, 1 minute after mixing, and 2 minutes after. During this period the opacity almost always falls in NaCl suspensions, and invariably rises in citrate-formol, and at the end of 2 minutes a steady state is reached. To get information about stirring effects, the tubes were inverted at the end of 2 minutes, or their contents stirred with a small air jet. This usually results in an increase in the opacity within 15 seconds after the stirring, and a return to the original steady value within a minute.

The red cell counts were made on the samples of heparinized blood in the usual way, 5 squares being counted on each side of a double chambered Levy-Hauser counting chamber, and checked by a second pair of counts in cases where the deviation of the first pair exceeded $2\sqrt{n}/n$.[4]

[3] Shohl used the Evelyn photometer, which is of small aperature.

[4] While the theoretical standard error for the distribution of n cells is $\sqrt{n}/n$, as shown by "Student" in 1906, deviations of more than twice the standard error occur in practice with an unexpectedly high frequency. It is also noticeable that the error does not diminish as rapidly as it should when larger numbers of cells are counted, i.e., it is somewhat like the standard error of the polynuclear count (Ponder, Saslow and Schweizer, 1931). Attention should be called to the fact that there are considerable errors connected with the process of sampling, for blood in a vial, even if well stirred, is not altogether homogeneous; this is probably due to the cells being carried, en masse, in shoals by currents in the fluid, and is what gives rise, essentially, to the stirring errors described in this paper. In practice, there must be added errors in-

The hematocrit tubes were 100 mm. long, about 1 mm. in bore, and made of heavy walled glass. They were filled by means of capillary pipettes, and spun at 3000 r.p.m. for 15 minutes; this may not be an ideal speed and time, but results in the attainment of constant volume, and is satisfactory, in my experience, *for comparative purposes.* In measuring the length of the column of packed cells, the buffy coat was not included; at the same time, however, the length of the white cell column was measured, and a white cell count was done in the usual way. Hemoglobin was also determined on each sample.[5]

Results. To express the relation between any pair of measurements (e.g., red cell count and opacity as measured with the red filter after 2 minutes, the cells being in citrate-formol), the figures for the count and for the opacity were plotted against each other, and a best line passing through zero was drawn through them by the method of least squares. For each opacity reading, the difference between the corresponding observed count and the count derived from the linear relation was obtained; the standard deviation σ of the deviations was then found in the usual way. This is better than calculating a coefficient of correlation between the two variables, because it gives a direct measure of the degree of scatter of the discrepancies and of the frequency with which a discrepancy of any given magnitude may be expected to occur. The values of σ for different pairs of measurements are shown in table 1. The magnitude and distribution of the stirring effects are shown in table 2, each stirring effect being expressed as a percentage of the photometer reading.

Discussion. As regards the correspondence between red cell counts and opacity measurements, the most satisfactory agreement is found when

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troduced by variations in shaking in the hemacytometer pipettes; we use a standard shaking, by hand, of 5 minutes, but a shaking machine is to be preferred.

[5] While the number of determinations made in the course of this investigation is not large enough for any very detailed statistical analysis, I have not been able to find any relation between the number of white cells present and the magnitude of the discrepancy between the observed count (or hematocrit reading) and the value given by the linear relation determined from the mass of the data. Shohl has pointed out that caution ought to be exercised in applying the opacity method to samples in which the number of white cells is greatly increased, and in this investigation I have rejected all observations for samples in which the total white cell count was less than 5,000 per mm^3. or more than 10,000 per mm^3. This means that the buffy coat did not exceed about 1 mm. in the hematocrit tube, and it is no doubt because of the restriction placed on the number of white cells allowable that no indication of their effect on the opacity appears in the figures. In some cases in which the white cell count was in the neighborhood of 20,000 to 40,000, the results obtained from the opacity measurements agreed very badly with those of red cell counting, as might be expected. Curiously enough, variations in the hemoglobin content have not been found to affect the discrepancy between opacimetry and red cell counting or hematocrit determinations, even when the green filter was used.

the cells are suspended in citrate-formol and when the opacity measurements are made with a red filter (maximum transmission 6600 A) and 2 minutes after adding the cells to the suspension medium.[6] Even under these circumstances, however, the standard deviation of the discrepancies between the count as made directly and the count as calculated from the opacity amounts to $\pm 0.545 \times 10^6$, i.e., about two-thirds of the discrepancies are less than this, but about one-third of them is greater.

At this point the question must arise as to the extent to which the discrepancies are contributed to by the red cell counts themselves being in error. The standard error which attaches itself to a red cell count by reason of the imperfection of the distribution of the cells on the squares of the chamber is $\sqrt{n}/n$ in theory ("Student", 1906), and somewhat greater

TABLE 1

Red cell count and opacity	Green	1st setting	NaCl	$\pm 0.511 \times 10^6$
	Red	1st setting	NaCl	$\pm 0.467 \times 10^6$
	Red	2nd setting	NaCl	$\pm 0.570 \times 10^6$
	Red	2nd setting	Citrate-formol	$\pm 0.545 \times 10^6$
Hematocrit and opacity	Red	1st setting	NaCl	±3.11 units
	Red	2nd setting	Citrate-formol	±2.70 units

TABLE 2

Distribution of stirring effects, per cent

	NEGATIVE	0-2	2-4	4-6	6-8	8-10	10+
NaCl	2	10	15	15	4	2	2
Citrate-formol	0	37	13	0	0	0	0

in practice (Ponder, Saslow and Schweizer, 1931). The average red cell count in the series for the red filter and citrate-formol was 4,270,000, and with 2 sides of the chamber counted, the number of cells seen, n, would be about 800; this gives a theoretical error of ±3.5 per cent. The standard error of the discrepancy between the count obtained directly and the count calculated from opacity, however, is ±0.545/4.27, or ±12.7 per cent, and so it is unlikely that the error of direct counting goes more than about halfway towards accounting for the standard error of the discrepancy. Errors

[6] Numerically the value of σ for the 1st setting with either the red or the green filter and a NaCl suspension medium is smaller, but not significantly so, and the stirring variations are more pronounced (table 2). All the stirring effects described here are quite different from the "spontaneous changes in light transmission" described by Kesten and Zucker (1928); the latter are observed after 2 to 3 hours, and are apparently due to the cells assuming the spherical form. They result in an increase in the opacity.

due to diluting and to obtaining a good sample of blood are common to both direct counting and opacimetry, and so cannot be held responsible.

Remarks of a similar kind apply to the relation between opacity and percentage volume as found by the hematocrit. Citrate-formol is again the preferable medium, and the red filter preferable to the green one, but the standard error of the discrepancies amounts to ± 2.7 units of volume. The hematocrit method may not be a very reliable one, but when used for relative purposes its standard error is certainly less than this.

There is every reason, indeed, to believe that the opacity of a red cell suspension is a function of several variables other than number and size (e.g., shape, refractive index differences, etc.), and perhaps the clearest evidence of this is that the correlation coefficient between the opacity as measured in citrate-formol and the opacity as measured in NaCl is only 0.9 ± 0.03, i.e., the two sets of measurements are not measuring quite the same thing, and there exist factors which do not influence them both identically. As Shohl points out, one should not lose sight of the fact that a disagreement between the results of red cell counting or the hematocrit on one hand, and of opacimetry on the other, does not mean that the former methods are necessarily right and the latter wrong, for the methods measure different physical properties of the blood and therefore all the results may be valid; the discrepancies, indeed, may themselves be significant, and may be correlated with as yet unidentified differences in the state of the cells. Further investigation is needed along these lines. The question nevertheless remains: Can the opacimeter method, in its present form, be used to replace red cell counts and hematocrit determinations for routine clinical purposes? Because of the size of the standard errors, I think that the answer must be no, for there is about 1 chance in 10 that opacimetry will give a difference of more than $\pm 500{,}000$ between two red cell counts when none exists. Direct red cell counting has always the advantage that any uncertainty can be removed by repeating the count so as to diminish the standard error, which can theoretically be reduced indefinitely by increasing n. For this reason it must remain the standard method.

The results do not throw much light on the nature of the stirring effects in suspensions, but several points emerge quite clearly. 1. The opacity of suspensions of red cells in citrate-formol is always greater than that of cells in NaCl, in part at least because the cells swell in citrate-formol (Shohl, 1940, Ponder, 1940). Whether the swelling is great enough to account for the opacity difference (opacity in citrate-formol = 1.48 times opacity in NaCl, on the average) is not clear. 2. With a very few exceptions, the stirring effects are always such as produce a greater opacity, and they are larger and more variable in NaCl than in citrate-formol. Their effect is just the opposite from the stirring effects met with in con-

ductivity work, where stirring produces a small decrease in resistance and capacity, or an increased conductivity (Fricke and Morse, 1925). 3. The effects on opacity and on conductivity are much greater than can be accounted for by changes in the orientation of individual cells. If we have a suspension of red cells in the path of a parallel beam of light or between two parallel electrodes, the opacity and resistance will depend, in a complex manner, on the effective area or "target area" which the cells present in in the direction of the light or of the current flow. To take extreme instances, all the cells might be oriented edge-on or all oriented face-on, and in the former case the opacity and resistance would be expected to be less than in the latter since the target area is smaller. In any real case the target area S will be somewhere between the two extremes. If S is the maximum target area of the single discoidal cell of radius r ($s = \pi r^2$), it can be shown (Cox, 1941) that $S = Ns/2$ and that its fractional mean square deviation is $1/3N$, where N is a number of cells oriented at random. This deviation is far too small to account for the effects of stirring on the basis of chance changes in the orientation of single red cells. We have rather to suppose that there is orientation of masses of cells on a large scale, the orienting forces being presumably currents in the suspension fluid (cf. Gorin and Velick, 1940). In this connection it may be remarked that in a suspension at rest the gravitational forces are not great enough to orient the cells in the position of greatest hydrodynamic stability, i.e., on the flat; stirring accordingly produces a change from a state of randomness to one of orientation, and not *vice versa*, as is often implied.

SUMMARY

Although the number of red cells and the percentage volume occupied by them can be found approximately by measuring the opacity of a suspension, the fact that the opacity is a function of variables other than number and size leads to results which often are in poor agreement with the results of direct counting and of hematocrit determinations. The standard error of the discrepancies between the count obtained by direct counting and that calculated from opacimetry is about ±500,000, and that of the discrepancies in percentage volume about ±2.7 units of volume.

A solution of isotonic sodium citrate with 0.1 per cent formol added is preferable to isotonic NaCl as a suspension fluid for opacity measurements, but only because the variations in light transmission which accompany stirring are greatly lessened. These stirring effects seem to be due to the orientation of masses of cells by currents in the suspension medium.

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SOME EFFECTS OF COBALT AND LIVER SUBSTANCE ON BLOOD BUILDING IN DOGS[1]

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Polycythemia was first produced in rats by cobalt feeding in 1929 by Waltner and Waltner (1). This observation has been confirmed with rats and extended to dogs, pigs, rabbits, guinea pigs, mice, frogs and chickens. Thus, cobalt seems to possess the general biologic property of stimulating erythrogenesis when fed in physiologically massive doses.

The etiology of polycythemia is little understood. Although cobalt feeding, lowered oxygen tension, or extended regular muscular exercise, all stimulate the development of polycythemia, their exact modes of action are not known. Orten (2) suggested that cobalt causes vasodilatation, thus producing anoxemia, which constitutes a primary hematopoietic stimulus. Mascherpa (3), who first reported cobalt polycythemia in dogs, observed hyperplasia of the bone marrow and considered the action of cobalt to be directly on the erythropoietic centers. Kleinberg, Gordon and Charipper (4) produced anemia in rabbits by bleeding and by benzol injection, and induced rapid erythropoiesis by injection of cobalt. They concluded that cobalt stimulates the formation of erythrogenic precursors in the bone marrow.

Recently Davis (5) and Brewer (6) have contested the fact of whether or not a true polycythemia is obtained uniformly and maintained consistently in dogs fed high levels of cobalt. Brewer (6) subjected his own and Davis' data to statistical analysis and concluded that cobalt feeding actually caused a depression in blood values in some cases, although in general it caused a slight increase.

A satisfactory theory for the action of cobalt, or, for that matter, any

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A preliminary report of this investigation was presented at the Toronto Meeting of the American Society of Biological Chemistry, April, 1939 (Frost, D. V. and C. A. Elvehjem, J. Biol. Chem. **128:** xxi, 1939).

[2] Now at Abbott Laboratories, North Chicago, Illinois.

causative factor of polycythemia is difficult to formulate with the facts at hand. Most reports of experimental work dealing with the condition fail to take account of the variable effect diet may play and it seems quite probable that this will need to be more fully understood and placed under experimental control before the picture of the physiological control of hematopoiesis can be assessed. In any condition, however, the possibility for uncontrolled variation is certainly large.

In studies extending over a period of five years in this laboratory the adequacy of milk diets with added iron, copper and manganese (7, 8) to support hematopoiesis in dogs has been demonstrated. Recently (9) the question of the need for cobalt for rapid blood formation was studied using this diet. Although some evidence indicated a stimulating effect of small amounts of cobalt on the rate of hematopoiesis, definite conclusions could not be drawn as to its essentiality. The adequacy of milk with added iron, copper and manganese was perhaps best shown by experiments (10) designed to compare the effect of iron and copper versus whole liver substance to support blood building. Copper and iron appeared to be about equal to liver in this capacity in these experiments. Thus there appeared to be no evidence for the existence in liver of special hematopoietic factors not supplied in adequate amount by milk. That liver does contain factor(s) qualitatively or quantitatively different from those in milk which can have a very profound effect on the hematopoietic function was discovered quite unexpectedly.

During the course of experiments (9) to determine the need for cobalt in dogs on milk diets we produced anemia in several adult dogs by phlebotomy. One of the dogs was fed a high level of cobalt about three weeks after severe anemia had been produced. The cobalt feeding had an apparent adverse effect on the appetite and general condition of the dog, but this was not surprising. However, the failure of iron and copper additions to initiate hematopoiesis after the cobalt feeding was indeed surprising, particularly in view of the fact that littermates which had been phlebotomized at the same time showed typical excellent responses to copper and iron alone. The continued failure of the dog on the cobalt, iron and copper regimen suggested the idea that cobalt feeding under these conditions might create an unnaturally large demand for certain hematopoietic precursors. The most likely material to test for such precursors was whole liver substance, and the response of the dog to liver feeding, despite continuation of the other therapy, was profound both as to the effect on general health and rate of hematopoiesis. The above experiment and further experiments of this type are described more completely below.

The finding that liver overcomes an apparent inhibition to hematopoiesis induced by cobalt stimulated our interest in the recent work of Davis who had reported results with cobalt and liver feeding which appeared in almost

direct antithesis to our results. Davis (5, 11) reported a stimulation of blood formation induced by cobalt feeding and a subsequent decrease when liver was added. In order to test the polycythemia producing potency of cobalt in dogs on milk diets we conducted the experiments described below. It was hoped that these experiments would serve also to throw some light on the controversial findings of Davis (5) and of Brewer (6).

EXPERIMENTAL. *Methods.* In the case of the adult dogs used in this study blood samples were obtained from the radial vein while blood from the younger animals was drawn from the jugular vein. In all cases 5 to 10 cc. of blood was drawn into the non-shrinking oxalate mixture described by Wintrobe (12). Hemoglobin, hematocrit and erythrocyte measurements were made within one hour after sampling. Hemoglobin concentration was measured by a method adapted to the Evelyn photoelectric colorimeter and the percentage volumes of packed red cells were determined by means of the Wintrobe hematocrit tube (13). Hayem's solution was used to dilute the blood for the erythrocyte counts which were made on a Spencer Bright-Line hemocytometer. Mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration calculations were made by the method suggested by Wintrobe (14).

Inhibition of hematopoiesis by cobalt feeding. The following experiments were carried out on four separate dogs at different times, but the methods used were comparable and the experiments can be described together. The dogs were all about one year of age and had received only milk with iron, copper and manganese from infancy. All of them had been cured of two successive anemias with copper and iron and were normal when the experiment was begun. Each dog was placed on a diet of milk alone and was bled periodically until a stable state of anemia was reached (Hb about 8 grams per 100 cc. blood). They were then given 4 mgm. daily of cobalt as a solution of cobaltous chloride. This was continued for two weeks without much apparent effect, except possibly to aggravate the anorexia already apparent due to the anemia. After two weeks of cobalt feeding, iron and copper were added at levels of 30 mgm. and 4 mgm. respectively daily. No beneficial effect was noted during the following two week period of iron, copper and cobalt feeding. In the case of dog 2, in which the effect was first noted, the iron level was increased to 60 mgm. daily, without apparent benefit to the dog. After two weeks of iron, copper and cobalt therapy, the supplements of the individual dogs were varied. Dog 2 was given 100 grams of whole dry liver and dog 4 was given 20 grams of liver extract (1:20 powder) in addition to the previous therapy. In each case an increase in appetite, weight and hemoglobin level ensued. The magnitude of the increases is seen in figure 1.

Dogs 6 and 13 were carried for a considerable period without any therapy in addition to the iron, copper and cobalt. Various supplements were

tried over a period of months and finally the dogs were brought to normal by the addition of liver fractions.

Production of polycythemia in adult dogs by cobalt feeding. The average blood picture of five adult dogs maintained on milk with added iron, copper and manganese is shown in table 1. The average hemoglobin level, erythrocyte count and hematocrit per cent of these dogs compare favorably with the averages found by Bruner and Wakerlin (15) for normal adult dogs. Addition of 3 to 6 mgm. cobalt per kilogram of body weight to the regular daily supplement of 30 mgm. iron, 3 mgm. copper and 3 mgm. manganese resulted in a large increase in blood values in the first few days

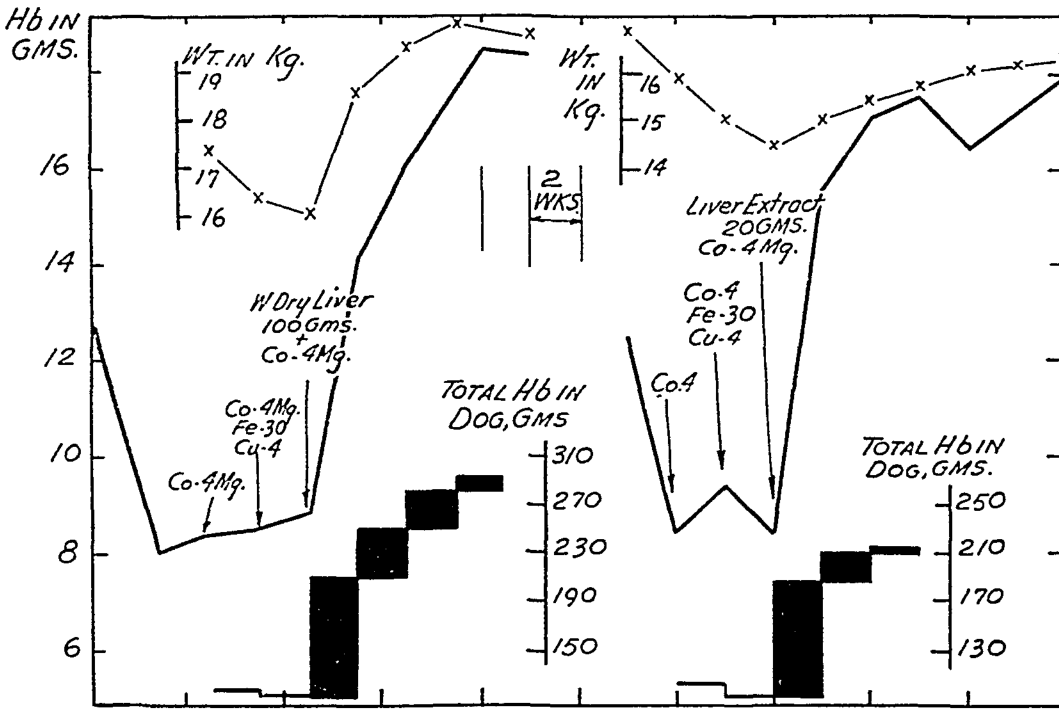

Fig. 1. The effect of liver and liver extract on anemia produced by bleeding and resistant to iron, copper, manganese and cobalt.

of cobalt feeding. The highest values obtained by this procedure were: hemoglobin 20.6, hematocrit 65, and erythrocyte count 11.6. Generally the blood values decreased gradually despite continued cobalt feeding until the blood picture was nearly normal at the end of three or four weeks. The fact that a clear-cut polycythemia was obtained during the first three weeks of cobalt feeding is shown in table 2. The calculated corpuscular values for the polycythemic bloods are probably not significantly different from those of the normal bloods.

The addition of 75 grams of fresh calves' liver, 25 grams of whole dried hog liver, 8 mgm. choline per kgm. of body weight, or 60 mgm. of crystalline vitamin C daily did not appear to cause a more rapid lowering in polycy-

themic blood values than naturally occurred over a period of 3 to 4 weeks. The addition of liver improved the general condition of polycythemic dogs as evidenced by weight gain and, if anything, caused the polycythemia to be maintained.

Effect of high levels of cobalt in young dogs. Considerable data have been obtained (7, 8, 9) on the normal rate of hemoglobin production in young dogs on milk diets with added iron, copper, manganese and traces of cobalt. These studies have revealed a slow prolonged rise to normal adult blood values during growth, a phenomenon common to most animal species.

TABLE 1

Blood picture of five adult dogs on a mineralized milk diet

Composite of a large number of analyses

MEASUREMENT	MEAN	MAXIMUM	MINIMUM
Erythrocytes, million per cmm	6.97	7.8	6.1
Hemoglobin, grams per 100 cc	14.29	17.5	11.5
Hematocrit, per cent	43.3	52	39
Mean corp. volume	62	83	55
Mean corp. Hb	21	26	17
Mean corp. Hb conc	33	34	27

TABLE 2

Typical average blood values in first three weeks of cobalt feeding in adult dogs maintained on milk plus iron, copper and manganese

INDEX	DOG 19	DOG 4	DOG 6
Erythrocytes, million per cmm	9.2	8.4	7.7
Hb, grams per 100 cc	18.1	19.8	16.9
Ht, per cent	55	58	52
Mean corp. volume	60	69	68
Mean corp. Hb	19.7	23.6	22
Mean corp. Hb. conc	33	34	33

This apparent physiological lag phase may be largely independent of the nutrition of the animal and we desired to study the effects of high levels of cobalt during this stage. As the following experiments show, cobalt at levels which produce transient polycythemia in adult dogs, does not stimulate blood production in young dogs and is furthermore quite toxic.

Puppies were raised for this experiment in customary fashion on adequate levels of iron and copper and milk *ad libitum*. At 15 weeks of age dogs 26, 27, 29 and 30 were fed daily 80 mgm. of cobalt as cobaltous chloride in addition to 10 mgm. of iron and 2 mgm. of copper. Blood indices showed no significant change over a two week period of therapy. At 17 weeks of age

the iron supplement was raised to 30 mgm. daily and 2 mgm. of manganese was administered. No significant change in blood values was noted over two weeks of therapy. However, toxic symptoms began to appear at this point. Loss of weight, anorexia and vomiting were noted. The dogs were taken off therapy at 19 weeks of age.

At 20 weeks of age dogs 26 and 29 were fed daily 30 mgm. of iron, 2 mgm. of copper, in addition to 2 mgm. of manganese and 10 grams of brewer's yeast. After two weeks of therapy, the blood indices showed no significant changes but the dogs returned to normal in respect to daily weight gains and appetite. At 22 weeks of age dogs 27 and 30 were fed daily 30 mgm. of iron, 2 mgm. of copper and 2 mgm. of manganese in addition to 40 mgm. of cobalt. The dogs maintained their weight and appetite but the erythrocyte count fell from an average of 5.7 to 4.9 million per cubic millimeter of blood. Also at 22 weeks of age dogs 26 and 29 were fed daily 40 mgm. of cobalt in addition to iron, copper, manganese and yeast. No toxic symptoms were noted and erythrocyte counts remained relatively constant. At 24 weeks of age dogs 27 and 30 were given daily supplements of 10 grams liver extract (no. 343) in addition to 30 mgm. of iron, 2 mgm. of copper, 2 mgm. of manganese and 40 mgm. of cobalt.

No significant changes in the blood indices of the dogs were noted during the periods of high cobalt feeding. The general condition of the animals improved when the cobalt therapy was suspended. The yeast and liver additions were perhaps too small to cause any significant changes in the blood picture in the presence of cobalt. Data for one dog in each pair are shown in table 3.

Discussion. The results of our experiments on the polycythemia producing effect of cobalt cannot be safely compared with those of Davis (5) and Brewer (6) because of the difference in basal diets used. The general agreement is made, however, that polycythemia can be produced in adult dogs by cobalt feeding. Our results indicate that this is not true for young growing dogs and that in adult dogs the effect is transient.

The ability of liver to overcome cobalt inhibition to normal hematopoiesis in dogs made anemic by hemorrhage is of interest because the notion has long been held that liver contains a substance which is concerned with the maintenance of the level of erythrocytes in the blood. Anderson, Underwood and Elvehjem (16) have stabilized cobalt polycythemia in rats by the addition of liver and liver fractions to mineralized milk diets. Davis has attributed a polycythemia depressing action to liver, and more specifically to choline (11) and vitamin C (17). Verzar (18) and Zih (19) have shown the erythropoietic and erythropenic action of bilirubin in rabbits. Barron and Barron (20) found that ascorbic acid prevents cobalt polycythemia in rabbits and suggest, therefore, that ascorbic acid is a regulator of red blood cell production.

Other experiments support the view that physical factors are important, at least in initiating erythrogenesis. Gordon and Kleinberg (21) have shown that guinea pigs subjected to low pressures develop erythremia, and their experiments indicate that when the bone marrow is stimulated excessively, it possesses a momentum which carries it beyond its normal limits of erythrogenesis.

TABLE 3

Blood changes in young dogs on high levels of cobalt

DOG	SUPPLEMENT	AGE	WEIGHT	Hb	Ht	RED BLOOD CELLS	Hb IN DOGS	MEAN CORP. Hb	MEAN CORP. VOL.	MEAN CORP. Hb CONC.
		weeks					*grams*			
26	Fe 10 + Cu 2 + Co 0.1	13	5.3	14.3	41	6.1	60	23.4	67	35
	Fe 10 + Cu 2 + Co 80	15	6.0	12.8	39	5.9	61	21.6	66	33
	Fe 30 + Cu 2 + Mn 2 + Co 88	17	6.0	12.9	39	5.8	62	22.0	67	33
	Off therapy (toxicity symptoms)	19	6.7	12.5	39	6.6	67	19.0	59	32
	Fe 30 + Cu 2 + Mn 2 + yeast 10 grams	20	5.5	13.7	44	6.8	60	20.0	65	31
	Fe 30 + Cu 2 + Co 40 + Mn 2 + yeast 10 grams	22	6.8	12.6	39	6.5	68	20.0	60	32
		23	7.7	12.2	40	6.2	75	20.0	65	30
		24	7.1	13.7	41	5.9	78	23.0	70	33
30	Fe 10 + Cu 2	13	6.0	13.5	39	5.6	65	24.0	70	35
	Fe 10 + Cu 2 + Co 80	15	6.7	13.9	38	5.2	74	27.0	73	37
	Fe 30 + Cu 2 + Mn 2 + Co 80	17	6.3	15.2	44	7.1	77	21.0	62	35
	Off therapy (toxicity symptoms)	19	5.8	16.0	47	7.6	74	21.0	62	34
		20	5.6	15.3	47	6.4	69	24.0	74	33
	Fe 30 + Cu 2 + Mn 2 + Co 40	22	6.8	12.9	38	5.3	69	24.0	72	34
		23	7.5	12.1	37	5.0	73	24.0	74	33
	Fe 30 + Cu 2 + Mn 2 + Co 40 + 10 grams L.E. #343	24	7.4	13.7	41	4.9	81	28.0	84	33
		25	7.35	14.7	44	5.4	86	27.0	81	33
		26	8.2	12.9	43	5.4	85	24.0	80	30

The failure of copper and iron to initiate hematopoiesis in phlebotomized dogs fed cobalt, and the profound response to liver strongly supports the idea that liver contains something other than iron and copper which is intimately concerned with stimulation of the blood forming centers.

Whether this substance is generally required in nutrition or whether it is of hormonal nature is not known. Preliminary evidence has been obtained that the pernicious anemia principle from liver is not involved. Vitamin B_6 appeared to cause some stimulation, but the response was not as great as that obtained with liver or liver extract (1:20 powder).

The importance of the ration in this type of study cannot be overestimated and it is questionable whether the results obtained with milk diets can be extended to other adequate diets. Although milk plus iron, copper and manganese supports a high rate of hematopoiesis in normal dogs, it differs in blood building properties from liver on a quantitative basis and possibly on a qualitative basis as well.

SUMMARY

1. An inhibition of the normal hematopoietic response to iron and copper feeding was observed in dogs made anemic by hemorrhage and fed cobalt prior to the addition of iron and copper. Hematopoietic activity was resumed on the feeding of whole dry liver or liver extract. The compound(s) in liver which cause this profound hematopoietic response have not been determined.

2. A temporary polycythemia was produced in adult dogs by feeding 3 to 6 mgm. of cobalt per kgm. of body weight in addition to the usual mineralized milk diets.

3. High levels of cobalt had a toxic effect on young growing dogs and little apparent effect on the blood picture.

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SHOCK FOLLOWING VENOUS OCCLUSION OF A LEG[1]

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In the course of experiments in which attempts were made to produce chronic edema the procedure of ligation of the iliac vein with injection of various materials distally into the vein was initiated. In a number of instances marked edema of the legs developed and associated with this the animals died within 24 hours, sometimes within 4 to 6 hours. The possibility of shock as the cause of death suggested itself and this was investigated in a series of 8 dogs.

METHOD. The dogs were anesthetized with ether. The common and internal iliac veins were ligated aseptically on one side using a retro-peritoneal approach; this was followed by the injection distally into the external iliac vein of 12 to 15 cc. of an autoclaved 1:20 suspension of lamp-black in physiological saline solution. The duration of the anesthesia was between 30 and 45 minutes. Several types of measurements were made at frequent intervals and compared with control observations made before the anesthesia and operation had been undertaken: *a*, arterial blood pressure measurements, using the Hamilton needle manometer technique (1), the needle being inserted in the contralateral femoral artery; *b*, heart rate, calculated from these records; *c*, hematocrit determination, using the method recommended by Scudder (2); however, blood was drawn from the femoral artery because of the difficulty of obtaining blood from the collapsed veins late in the course of the experiments; *d*, total plasma proteins, determined by the Kjeldahl method (3); *e*, rectal temperature; *f*, respiration, by timing with a stopwatch; *g*, measurements of the limb circumference of both hind legs as a crude guide to the development of edema. After the death of the animal, each of the hind limbs was disarticulated at the symphysis pubis and hip-joints, being sure to include the gluteal muscles, and then weighed.

RESULTS. The leg with the occluded veins became markedly enlarged and colder than the contralateral limb. The animals became listless and quiet, even during the manipulations of taking the various readings. Respirations usually increased in rate, especially toward the end when they became shallow and panting. Retching and vomiting usually occurred

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terminally, as well as cycling and spasmodic movements. Death was respiratory, cardiac standstill being delayed for several minutes.

There was no significant change in the rectal temperature throughout the experiment.

The changes in the total plasma protein, from blood taken at the beginning and at the end of the experiment, were not marked and probably within the experimental error of the determination. In 2 dogs total protein determinations were made at frequent intervals during the course of the experiment. Since only slight alterations were observed, it would appear that the capillaries of the limb with occluded veins lost their impermeability to the blood proteins early. The fluid lost from the blood therefore appears to be practically a plasma.

The blood pressure fell progressively to shock level; at first this affected the systolic and pulse pressure more than the diastolic. In part this was due to the marked acceleration of the heart, in part to the decrease in blood volume reaching the heart due to a loss of plasma as shown by the hematocrit. In the two animals which survived longer, there was a tendency for the blood pressure to rise in the last hour before death (fig. 1).

The heart rate increased after the operation and remained elevated until shortly before death when a final slowing occurred. In three of the animals slowing was noted to occur in the last hour of life.

In all instances there was a progressive hemoconcentration as measured by the hematocrit, and usually there was a terminal tendency for this to return toward normal. This hemoconcentration indicates a loss of plasma fluid into the edematous leg. The factors involved in the final decrease in the hematocrit appear to be:

1. A movement of fluid from other extracellular spaces to replace that lost into the edematous limb.
2. The increase in tissue pressure in the edematous leg lessening the movement of plasma out of the blood stream.
3. The development of hemorrhages.

The increase in the weight of the edematous leg over the contralateral one was remarkable as shown in table 1. The values range from 4 per cent to 6.1 per cent of the body weight. It is significant to note that there was a rough inverse ratio between the relative amount of fluid lost in the leg and the duration of life after operation. The loss of fluid thus amounted to approximately 50 per cent to 75 per cent of the usually accepted blood volume. It follows that this would cause not only the blood hemoconcentration found and a sharp drop in the circulatory blood volume but would of necessity draw upon the extracellular fluid reservoirs. This implies that one is dealing with a state of body dehydration and the conditions resulting from it.

Figure 2 represents one of the experiments in which muscle biopsies were

taken from the edematous leg. The histological sections are shown in figure 3. From such studies, the following sequence appears to have occurred. At first, slight distention of the capillaries was revealed with the presence of edema fluid in their vicinity. Later, the distention of the

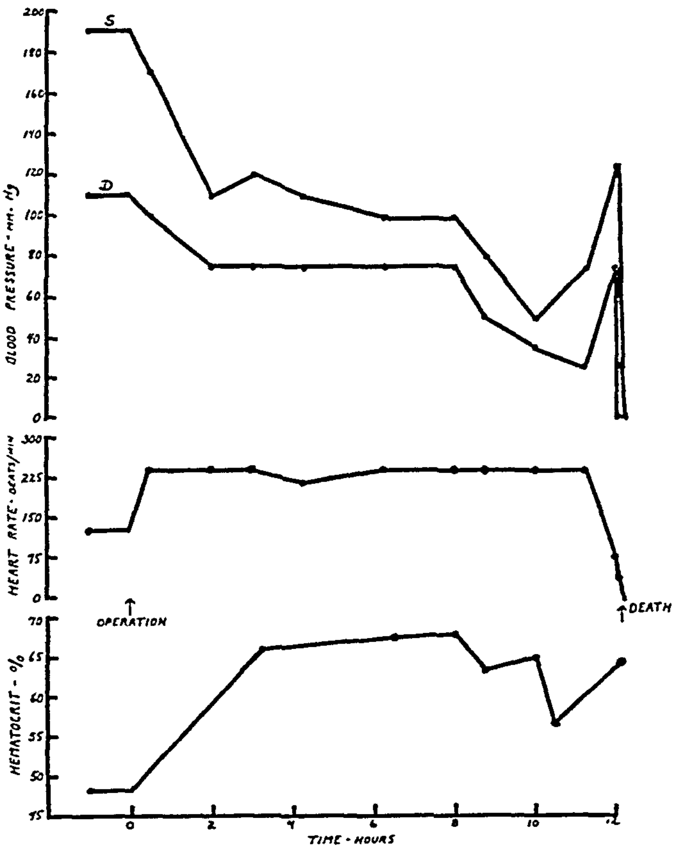

Fig. 1

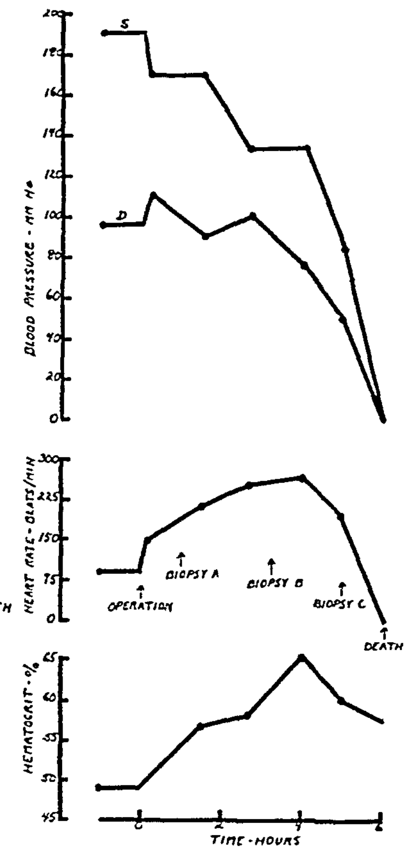

Fig. 2

Fig. 1. A typical chart of events following almost complete venous occlusion in one hind limb. S = systolic blood pressure; D = diastolic blood pressure. Weight of dog, = 12.7 kgm. Weight of disarticulated left leg (with venous occlusion), = 1.567 kgm. Weight of disarticulated right leg (control), = 1.064 kgm. Difference in weight, = 0.503 kgm.

Fig. 2. Another typical experiment, conventions as in figure 1. Weight of dog, = 13.6 kgm. Weight of disarticulated left leg (with venous occlusion), = 2.305 kgm. Weight of disarticulated right leg (control), = 1.568 kgm. Difference in weight, = 0.737 kgm. Muscle biopsies were taken from the leg with venous occlusion at the times indicated, and these, as well as sections taken at autopsy, were examined histologically.

capillaries and veins became more pronounced. When the dog was in shock, red blood cells appeared between the individual muscle fibers and in the intermuscular septa, and recent thrombi containing numerous lampblack particles were seen in the veins. The hemorrhages became progressively greater until death supervened.

TABLE 1

WEIGHT OF ANIMAL	INCREASE IN WEIGHT OF EDEMATOUS LEG OVER CONTRALATERAL ONE		DURATION OF LIFE AFTER OPERATION
kgm.	*grams*	*per cent body weight*	*hours*
10.9	668	6.1	4½
9.5	570	6.0	5
11.8	576	4.9	5¼
13.6	737	5.4	6
13.2	702	5.3	6½
15.4	750	4.8	9½
10.5	458	4.3	10
12.7	503	4.0	13½

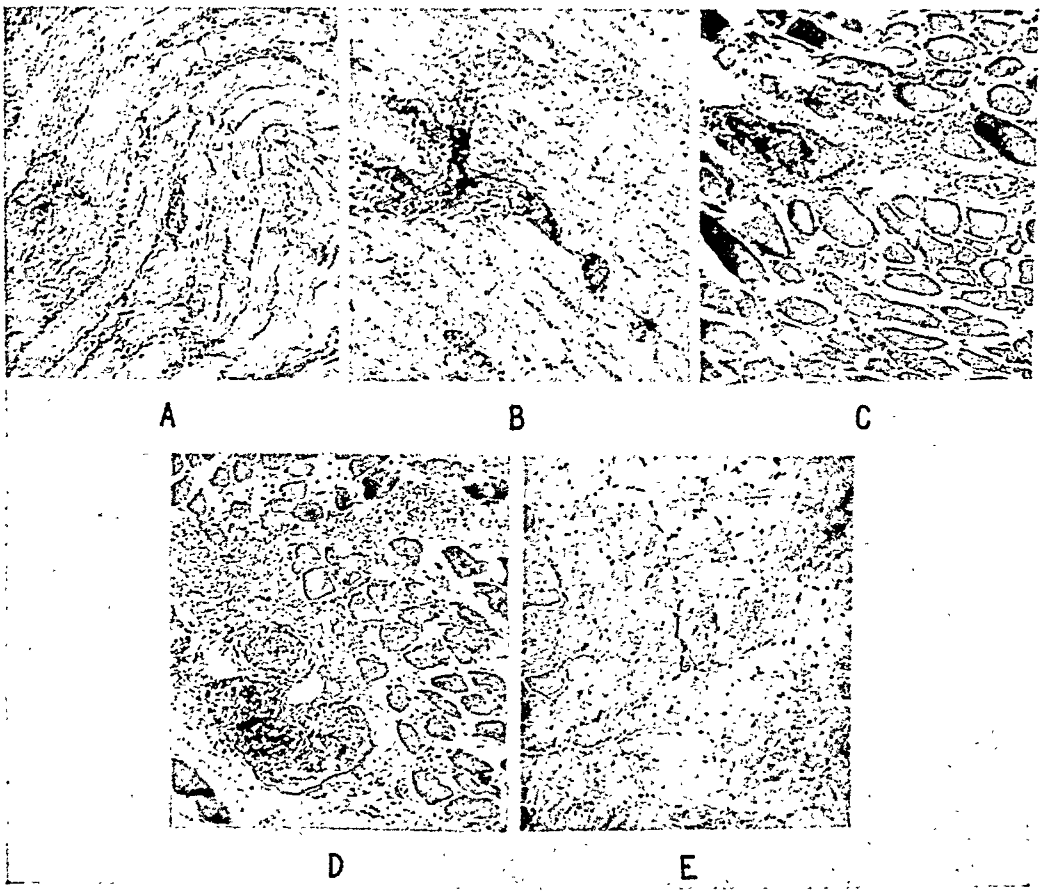

Fig. 3. Photomicrographs of biopsies of leg with venous occlusion, *A*, *B*, *C*, and of autopsy section from this leg, *D*, and from the contralateral control leg, *E* (Weigert's iron hematoxylin-eosin stain; × 110). Segment *A* shows in upper left hand corner slight dilatation of capillaries with surrounding edema. Segment *B* shows marked dilatation of the capillaries and veins. Segment *C* shows red blood cells between the individual muscle fibers and in the intermuscular septa; the vein contains a recent thrombus in which are numerous lampblack particles. Segment *D* shows even more hemorrhages between the muscle fibers and in the intermuscular septa, as well as a recent thrombus in the vein. Segment *E* shows no histopathologic changes.

Post-mortem examinations of the other organs showed several definite changes. The suprarenal glands revealed varying degrees of hyperemia and occasionally microscopic hemorrhages. These hemorrhagic areas were confined to the zona fasciculata and contained many polymorphonuclear leukocytes. There was marked dilatation of the central veins and sinusoids of the liver, and occasionally hyperemia of the capillaries of the lung and intestinal mucosa. The brain removed from one of the animals was very pale, and there was no gross or microscopic evidence of capillary dilatation or hemorrhage. The remaining organs presented no significant changes.

Discussion. These experiments illustrate that an extremely large amount of fluid can be accumulated in a limb when its venous channels are fairly completely occluded; sufficient, in fact, to cause hemoconcentration, a drop in blood pressure to shock levels and symptoms and signs of shock terminating in death. It is possible that on rare occasions an extensive thrombophlebitis of the common iliac vein in man, developing very rapidly, may lead to death in a like manner. Such appeared to be the case in a patient recently reported by Phemister (4). A woman developed a rapidly increasing swelling of one leg and died within 24 hours in shock. At autopsy a fresh thrombophlebitis completely blocking the common iliac vein and partly blocking the inferior vena cava was found. From measurements of the lower limbs, it was calculated that the volume of the limb with the thrombosis was about 4 liters greater than the contralateral one so that the immediate cause of death appeared to be due to the great loss of plasma into the occluded limb.

The procedure carried out in these experiments seems to offer a simple, certain method of inducing a state of shock and so lends itself readily to the utilization of the study of the sequence of events that occur and as a simple procedure to test the efficacy of some forms of suggested shock therapy.

It illustrates the magnitude of fluid loss from the rest of the body which can be accomplished by such a procedure and lends support to the most widely accepted view held at present, namely, that such localized fluid loss is the primary, or, at least, the most important factor involved in the production of shock (5). Apparently, in these experiments it was the fluid loss per se that established the chain of events leading to shock and early death.

The dynamics involved in the accumulation of the fluid locally are apparent. The ligation of the major veins of the limb by themselves, we have found, does not cause edema, or only a slight and transient edema. Apparently there is a widespread reserve of collaterals that quickly compensates for the occlusion and permits the blood drainage to be quickly reestablished. The injection of lampblack seems to form foci for the forma-

tion of thrombi resulting in the plugging of the majority of these collaterals. As a consequence, drainage of blood is slowed down markedly. The blood vessels in the leg, therefore, change quickly from a hydraulic system with blood in motion to a practically hydrostatic one with little flow. This implies that the gradient of pressure normally existent from the arteries to the veins disappears to a large extent. As a result, the pressure in the capillaries will tend to approach the pressure in the arteries and this would cause the hydrostatic factor to increase over the oncotic and thus lead to the pouring out of fluid into the leg tissues. Two circumstances would tend to limit this fluid escape. The first is the increase in tissue pressure; the second, the increase in oncotic pressure as fluid escapes and blood proteins do not. However, the stasis itself would quickly alter the permeability of the capillaries and so permit the protein also to escape, as our blood protein studies suggest. In fact, this damage to the capillaries soon becomes sufficient to allow the escape of blood and so lead to the hemorrhages, which were actually found to be extensive.

As a result of this fluid loss the entire picture of shock developed and led to the animals' death.

SUMMARY

1. Nearly complete venous occlusion of a hind limb of the dog leads to shock which terminates fatally.

2. This procedure offers a simple way of studying the course of shock and the utility of some of the proposed therapeutic agents to counteract shock.

3. The mechanism appears to be the marked loss of fluid into the leg, at first plasma and later whole blood, which amounts to from 4 to 6 per cent of the body weight. This loss is brought about first by an increase in the capillary hydrostatic pressure of the occluded limb soon aggravated by loss of capillary permeability.

4. These experiments tend to support the view that the primary mechanism in shock is the local loss of fluid from the blood.

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Addendum. Since the communication was sent to press 5 more animals were tested and in general the relation of duration of life after the operation to the percentage loss of body fluid into the limb followed the same trend evidenced in table 1.

THE ACTION OF EXERCISE ON KETOSIS

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In recent years evidence has been accumulating that the ketone bodies are, on occasion, an important step in the catabolism of fat (1, 2). There is much support for the view that the liver at certain times partially oxidizes fatty acids to form these ketone bodies which are then supplied to the other tissues of the body as an important fuel (3, 4). A large fraction of the energy turnover of the muscles may under certain conditions be supplied by oxidation of these substances (1, 5). It would seem likely then that individuals in a state of ketosis would show a decrease in its intensity (which has ordinarily been measured by urinary ketone excretion) as a result of exercise. This has not been found to be entirely the case for periods of exercise lasting an hour or more (6, 7). These seemingly contradictory findings may be reconciled if we can accept the assumption already stated in papers from these laboratories (1) that muscular activity not only causes increased burning of these bodies but also increased production of them by the liver.

If these two effects are produced at the same time they would tend to balance each other and it would be very difficult to investigate them separately. If they are not simultaneous,—one being delayed—, it should be possible to follow the successive opposite effects. The increased utilization of ketone bodies by working muscles is immediate as is evident from the observations of Blixenkrone-Moller (5) on perfused isolated extremities and of Drury and Wick (8) on the intact subject. If there is an increased output by the liver and it has a delay of over half an hour it should be possible to follow it since it should give a post-exercise increase in the blood ketone curve. With the human subject we experienced difficulty in producing a "steady" ketosis state on which we could study this delayed effect of exercise. One can easily produce a definite ketonemia by a fast of 20 hours, but at this time the level is not constant, but has a distinct, though not necessarily steady upward gradient. Over a three day period this averages 0.82 mgm. per cent per hour (9). Our subject showed a gradient of 2.2 mgm. per cent per hour between the 10th and 20th hours. Under these conditions it is easy to study mechanisms which lower the

ketone content of the body, but it is difficult to follow those which increase it. One is forced to attempt to determine whether the post-exercise blood ketone curve has a steeper gradient than a control curve for the subject during the same period of fasting.

HUMAN EXPERIMENTS. In all of our human experiments we produced ketonemia by having the subject go without food for at least 15 hours. This was easily carried out by having him take no food after the evening meal of the previous day and then starting observations at 9 a.m. or later. At this time the blood ketone level is beginning to rise and continues to do so for many hours (8). We then had the subject exercise or rest for varying periods to see how this upward gradient of blood ketones would be affected during the exercises and during the period which followed. Blood ketone

TABLE 1

DAY 1		DAY 2	
Time	Blood ketones	Time	Blood ketones
	mgm. per cent		*mgm. per cent*
9:25 a.m.	1.8	9:15 a.m.	1.2
Walking		Rest	
10:50 a.m.	2.5	10:20 a.m.	1.2
Walking		Rest	
11:50 a.m.	1.6	11:05 a.m.	1.3
Rest		Walking	
1:10 p.m.	6.0	1:10 p.m.	3.4
Rest		Rest	
3:15 p.m.	8.9	2:15 p.m.	4.8

determinations were done by the method of Barnes and Wick (10) and are expressed as total ketone bodies.

Light exercise. The first exercise studied was walking. This was studied on two separate days a week apart. Every attempt was made to keep conditions identical on the two occasions (character of food on previous day, times of eating and sleeping) and the only thing that was varied was the times of walking and resting.

Although the results of the first day might suggest an increase in utilization during walking with an increased production in the supervening rest period, the second day shows the same general upward trend at any comparable period regardless of whether the subject rested or walked, or had been walking just before. We may conclude then that if walking does increase the utilization of ketone bodies it does so to but a small extent, and this small increase would be compensated for by increased production by the liver.

Moderate exercise. A heavier form of exercise was next studied—tennis playing. Table 2 shows the results of our first experiment with this.

It is apparent that playing tennis for this period has little demonstrable effect on the course of the blood ketone level. Its steadily rising trend may have been slightly flattened during playing. We, therefore, examined the effect of this form of exercise when continued for a longer period. Table 3 gives the results together with a control day on which conditions were identical except for exercise.

It would seem that exercise might have leveled out the upward trend of the blood ketones during the first hour and a half, but not after this. The rise between 11:04 and 12:20 may represent increased production by

TABLE 2

TIME	BLOOD KETONES
	mgm. per cent
3:20 p.m.	5.5
4:20 p.m.	6.4
Tennis playing	
5:10 p.m.	6.6
7:05 p.m.	12.7

TABLE 3

EXERCISE DAY		CONTROL DAY	
Time	Blood ketones	Time	Blood ketones
	mgm. per cent		*mgm. per cent*
9:37 a.m.	6.7	9:40 a.m.	6.0
Tennis		11:40 a.m.	8.2
10:13 a.m.	5.9	1:00 p.m.	19.7
Tennis			
11:04 a.m.	6.3		
Tennis			
12:20 p.m.	12.8		
Rest			
2:05 p.m.	19.5		

the liver. The differences from the control period, however, are so slight that it appears again that in moderate exercise one cannot demonstrate increased utilization of ketone bodies followed by increased production of these substances. Both may be increased but if so they offset each other very closely.

Heavy exercise. With a higher rate of exercise—running steadily at 10 miles per hour for 20 minutes—we obtained a definite decrease in the blood ketone level. This drop was followed by a sharp rise during the period after the exercise. See tables 4 and 5.

The examples show the drop during the heavy exercise and the sharp rise immediately after. The control days, however, have periods during

which the blood ketone level rises just as steeply. We can conclude then that a short bout of heavy exercise can use up ketone bodies faster than they are being formed by the liver. It is not possible to tell whether the succeeding rise due to liver production is greater than what would ordinarily occur because the control periods show at times rises just as steep.

RAT EXPERIMENTS. It seemed to us that the occurrence of these sharp rises in ketonemia on the control days make it very difficult to find out whether there is an over-production of ketone bodies by the liver after exercise in the human. We decided then to study an animal that had a

TABLE 4

CONTROL DAY		EXERCISE DAY	
Time	Blood ketones	Time	Blood ketones
	mgm. per cent		*mgm. per cent*
11:40 a.m.	8.2	3:40 p.m.	11.8
1:00 p.m.	19.7	4:40 p.m.	15.7
4:00 p.m.	22.9	Exercise	
		5:02 p.m.	9.6
		6:10 p.m.	16.2
		7:15 p.m.	18.8
		8:30 p.m.	20.3

TABLE 5

CONTROL DAY		EXERCISE DAY	
Time	Blood ketones	Time	Blood ketones
	mgm. per cent		*mgm. per cent*
12:10 p.m.	2.1	2:25 p.m.	4.3
3:20 p.m.	3.5	3:25 p.m.	7.7
5:25 p.m.	8.0	Exercise	
		3:45 p.m.	3.7
		5:00 p.m.	7.5

more slow and gradual rise in blood ketones during simple fasting (11, 12). The rat, which as an example may have an average rate of rise in the blood ketone level of 0.2 mgm. per cent per hour between the zero and ninety-sixth hour of fasting, satisfies this requirement. Even more important for the present problem, it is possible to fast these animals for periods adequate to arrive at a state in which the blood ketone level reaches a plateau and remains practically constant (13).

We made observations on 3 series of rats. For each series we selected a group of adult rats of common origin, of the same sex, approximately the same weight and within thirty days of the same age. They were all fasted

the same length of time, and then divided into two parts—one for the exercise and one for the control. The rats of the former group were given a short period of strenuous exercise (swimming) and at set times thereafter sub-groups of six were sacrificed for individual blood ketone determinations upon arterial blood. Similar sub-groups of six of the unexercised group were sacrificed at set times for control blood determinations. In all cases the liver glycogen content was also determined. Male rats were used in experiments A and B. In experiment A the rats (average body weight 281 grams) were used 48 hours after their removal from the stock diet ("Tioga dog Pellets", protein, 23; fat, 4; fiber, 4; ash, 12.5; moisture, 8.5; nitrogen free extract, 48). The rats in experiments B (average body weight 300 grams) and C (average body weight 202 grams) were on a low protein diet (13) for 15 and 16 days respectively and then fasted 3 days before using. Such a diet leads to a high fasting concentration for the blood acetone bodies (14).

The animals were exercised by swimming them in water (32°C) for five 2 minute periods with 1 minute rest between periods. With this amount of exercise the rats are completely exhausted and further swimming is generally impossible. The blood ketones were determined by the method of Barnes and Wick (10). Oxalated blood specimens were obtained from the abdominal aorta after the animals were anesthetized with sodium pentobarbital. Glycogen determinations were carried out on the livers according to the method of Good, Kramer and Somogyi (15).

The results are given in figure 1. Each point in a given experiment represents the average of the determinations for a group of six rats. The rats used in experiment A were evidently not fasted long enough to give a very high beginning blood ketone level, so the drop after exercise is not large. The subsequent over-production is quite definite. In the next series (B) we, therefore, made sure of an adequate beginning blood ketone level by feeding of a low protein diet and a longer fast. We also extended the time of post exercise observations. In this series we obtained a marked drop in blood ketone level immediately after the exercise, with a return to the control level one hour later. This is followed by an over-production phase, so that the level is much higher than the control two hours thereafter. At six hours after exercise the level has come back to that of the controls. In series C we produced a still higher beginning level, extended further the post-exercise period and determined the blood ketones every hour after the exercise. The results are even more pronounced than in experiment B. Two hours after exercise the over-production phase has not only made up for the immediate drop but has taken the level to well above the control. From then the level continues to rise slightly until the fifth hour. At the sixth and seventh hours the level is back to the control.

The changes in liver glycogen content pictured in figure 1 represent

weighted averages of the data for experiments A, B and C in which the changes were of the same general nature. The liver glycogen level was fairly low to begin with because the animals were fasting. During the brief exercise period it fell to a really low level and remained there throughout the period of observations. This is not surprising for in these glycogen depleted rats the only possible source of additional carbohydrate would be from protein catabolism. Although it has often been questioned, the old experiment of Pettenkofer and Voit (16), who found that in starvation exercise did not increase protein metabolism, has not been disproved. The energy for the exercise must be supplied by an increased metabolism

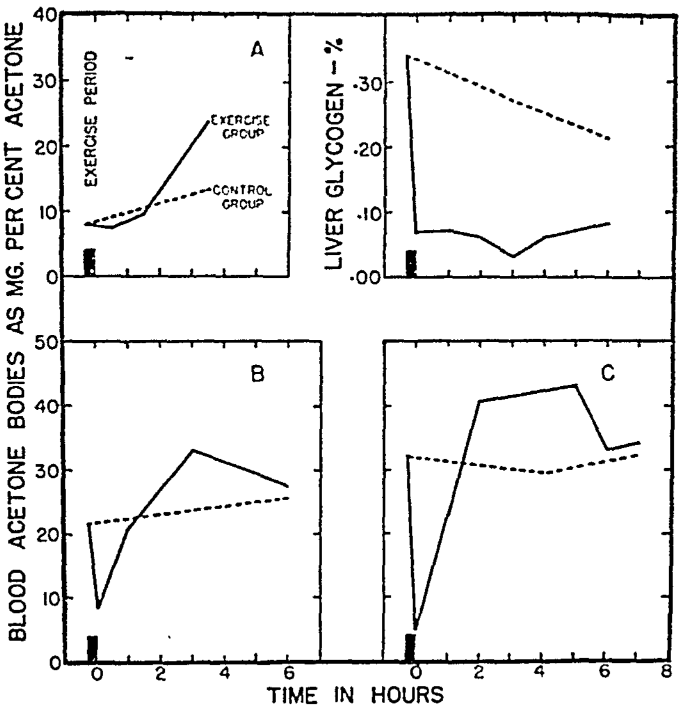

Fig. 1

of fat. The ketone bodies during exercise, when fasting, serve in part at least the functions of glucose during exercise in the fed organism (17–20).

Discussion. The results with the rat show that we can demonstrate clearly both increased utilization, and subsequent over-production of ketone bodies as a result of strenuous exercise in this animal. Heavy exercise also gives clear evidence of increased utilization with the human, and it is likely that a subsequent over-production would be clearly shown if we had fasted the subject until he had reached the blood ketone plateau. Evidently, demonstration of the two processes would be difficult for lower rates of exercise. Here the intensities of the two effects are less and in order to get the definite drop in blood ketone level that is needed to prove

increased utilization the exercise has to be continued for such an extended period that we get overlapping of the increased-production mechanism, and in this way the results of the two actions are balanced and consequently hidden.

Our results solve the problem presented by the finding of Blixenkrone-Moller that increased work by isolated muscles increases utilization of ketone bodies whereas increased muscular activity by the intact ketonic animal does not decrease the urinary excretion of ketone bodies in the 24 hour period. If the rate of exercise is high there is a reduction of ketones in the body and urine at the time, but this is compensated for by over-production during a period of several hours following the exercise, so that the total excretion during the two periods is little changed from control periods. It is reasonable to assume that the same mechanisms account for the absence of effect of less strenuous exercise continued for longer periods. Here the supervening increased production would commence before the increased utilization due to the exercise had continued long enough to have had a demonstrable effect. Although we can only get a separation of the two effects with short strenuous exercise, we have every reason to believe that they occur in milder forms of activity, here co-existing and offsetting each other.

A word should be said as to the possible mechanism of the action of exercise on ketosis. The initial fall in the ketone body level of the blood which results from exercise is most reasonably explained by an increased rate of utilization. What then of the subsequent overproduction? In severe exercise epinephrine secretion is abundant (20, 21) and causes an increase in glycogenolysis when carbohydrate for such is available. When there is a lack of carbohydrate, epinephrine increases ketone body formation (22). This may well be the cause of the over-production of ketone bodies during and immediately after severe exercise. Epinephrine may be an agent for increasing the production of ketone bodies when there is a condition that may require a lot of them (carbohydrate lack), similar to the way in which it increases glucose production when there is a need for it and an ample supply of glycogen is available.

SUMMARY

1. With rats, in a state of ketosis, a short bout of heavy exercise causes an immediate drop in the blood ketone level. During a period of three to four hours thereafter there is a phase of over-production of ketone bodies so that the blood values for exercised animals go to higher levels than in controls.

2. These results support the view that in ketosis states exercise increases the oxidation of ketone bodies and also causes the liver to produce them at a higher rate.

3. In man these changes were not demonstrated for light exercise. The drop during heavy exercise was obtained.

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FETAL ASPIRATION OF AMNIOTIC FLUID[1]

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The classical concept that the fetus in utero is apneic has from time to time been challenged and some investigators have declared that respiratory movements occur regularly throughout the latter part of prenatal life and represent normal physiological adjustments. On the basis of observations made on rabbits, Snyder and Rosenfeld (1) believe that the fetus in utero has a definite respiratory cycle and that amniotic fluid is normally drawn into the alveoli of the fetal lungs. They hold further that intra-uterine breathing is of functional significance in the development of the normal lung and serves to assist in the dilatation of the alveoli and elastic passages of the future air passages. Contrary to this conception are the observations made by Windle and his associates (2) on fetal goats, guinea pigs and cats. These investigators conclude that respiratory movements occur *in utero* only when abnormal physiological conditions (such as anoxia or hypercapnia) occur in the fetus. Clinical observations on the human fetus and new-born have led most obstetricians to consider the excessive inspiration of amniotic fluid as a predisposing factor toward pneumonia or other respiratory infections in the new-born. A high proportion of autopsies on new-born report the presence of amniotic sac contents in the lung. Thus, no one doubts that mammalian fetuses are capable of performing respiratory movements. However, the physiological normality of this process has not been established. In fact, there is clinical evidence that the aspiration of amniotic fluid into the alveoli of the lungs may result in death.

The following experiments were performed on rats in an attempt to determine the effect of inspiration of amniotic fluid on viability.

EXPERIMENTAL. On the nineteenth or twentieth day of pregnancy, the

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maternal rat was anesthetized with ether and the uterus exposed through a mid-line incision. One-half cubic centimeter of a warm sterile suspension of carbon in physiological saline solution was injected into the amniotic sac of each fetus directly under the fore leg. Care was taken to avoid injury to the placenta or to any of the uterine vessels when making the injection. After replacing the uterus, the animal was sewed up and delivery followed within 24 to 48 hours. All animals delivered dead were immediately removed and autopsy performed. All animals born alive were killed after 24 hours and autopsied. All lungs were removed and examined for the presence of carbon particles. When the lungs appeared pink or light grey, histological sections were prepared and a microscopic examination was made for the presence of carbon particles in the alveoli.

TABLE 1

	CARBON INJECTIONS		SALINE INJECTIONS
	Experiment I	Experiment II	
Number of fetuses injected	349	182	162
Number born dead	258	144	40
Percent of total injected born dead	69*	79†	25
Number dead within 24 hours (including still births)	279	166	68
Total mortality rate (per cent)	80	91	42
Animals living to 24 hours	70	16	87
Per cent of animals surviving at 24 hours with carbon in lungs	4	13	
Per cent of total animals injected living at 24 hours	20	9	58

* One hundred per cent of the 258 still-borns showed carbon in lungs.
† Ninety-three per cent of the 144 still-borns showed carbon in lungs.

In experiment I, 349 fetuses were injected with a suspension of carbon prepared by centrifuging a 1:1 dilution of Higgins' India Ink at 2,200 r.p.m. for 8 hours. The supernatant fluid was poured off, diluted with an equal volume of water and again centrifuged. This procedure was repeated twice until the ink was diluted to approximately one-quarter its original concentration.

In experiment II, a suspension of lamp black in 1 per cent gelatine was used for the injection. The carbon suspensions produced in this manner were non-toxic when injected intraperitoneally into 21-day-old rats.

A similar series of animals was run in which normal saline alone was injected into the amniotic sac.

Results. The results of the experiments are shown in table 1. In experiment I, 69 per cent of the 349 fetuses injected were still-born.

In experiment II the proportion of still births was 79 per cent.

In both experiments carbon particles were found in the lungs of practically all of the still-born animals (100 per cent in expt. I and 93 per cent in expt. II). If the animals which failed to survive 24 hours after birth are included, the mortality rate rises to 80 per cent in experiment I and 91 per cent in experiment II. Of the 86 animals which were still alive 24 hours after birth, only 5 (or 6 per cent) showed any trace of carbon particles in the lungs on autopsy.

When normal saline was injected into the amniotic sacs, only 25 per cent of the animals were still-born, and the total mortality rate at twenty-four hours was only 42 per cent, as compared with 80 per cent and 91 per cent in the carbon injection experiments.

Discussion. The results of these experiments indicate clearly that aspiration of large amounts of an amniotic fluid containing solid particles is fatal to the fetus. It may be that the stimulus to the aspiration was the anoxia and hypercapnia resulting from the operative procedures. However, the normal animals, as evidenced by their survival to 24 hours after birth, had inspired no amniotic fluid (or at best very little) under the same conditions, since their lungs showed no carbon particles.

Since the mortality rate[2] was 42 per cent when saline was injected, it is clear that death of the fetuses in experiments I and II cannot be attributed entirely to the presence of foreign particles (carbon) inspired into the lungs with the amniotic fluid. The high mortality rate found with the saline injections means that under the abnormal fetal conditions produced by the experimental procedure, amniotic fluid may be aspirated in sufficient amounts to result in the death of the fetus.

Summary. A suspension of carbon particles was injected in the amniotic sacs of 431 rat fetuses one to two days *pre partum*. Approximately 75 per cent were still-born and only 15 per cent were living 24 hours after birth. In contrast, 58 per cent of a group of 162 fetuses in which normal saline was injected into the amniotic sac under similar conditions were living 24 hours after birth. Over 90 per cent of the still-born animals showed carbon particles in the lungs when autopsied. Since some animals did survive 24 hours after birth and showed no carbon in the lungs, it cannot be contended that aspiration of amniotic fluid is a normal physiological process. The high incidence of still-births in both series leads to the conclusion that the excessive respiratory stimulation resulting from the fetal anoxia and hypercapnia attendant on the anesthetic and operative procedure caused the animals to inspire amniotic fluid.

[2] The expected still-birth rate in rats is given at 2 per cent by Donaldson.

CONCLUSIONS

1. Not all fetuses aspirate amniotic fluid into the lungs even under adverse physiological conditions.
2. Excessive inspiration of amniotic fluid will result in the death of the fetus.
3. It is highly improbable that amniotic fluid is normally drawn into the alveoli of the lungs in the fetus.

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THE TRANSPORTATION OF ABSORBED LIPIDS[1]

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In 1891 Munk and Rosenstein (12) reported the recovery from a chylous fistula of 60 per cent of the fat ingested by the subject. Since that time this figure has acquired an importance out of all proportion to the character of the experimental work upon which it was based, the assumption having generally been made that it actually represents the fraction of ingested fat absorbed into the circulation via the lymph. This has never been confirmed, Eckstein (5), for example, being able to recover from thoracic duct lymph only 4 to 21 per cent of the fat administered to dogs. Both in the original work and in subsequent attempts to confirm it, values have been referred to administered rather than absorbed fat, the fraction of the administered fat that was actually absorbed not having been determined. Hence, it is possible that *all* of the fat that was absorbed entered the lymph.

By studying the changes in blood lipid concentration before and after ligation or cannulation of the thoracic duct or of the portal blood before and after administering lipids, many attempts have been made to account for the 40 per cent that Munk failed to recover from the lymph. The results are inconclusive.

In the work reported here, we have measured the actual amount of administered lipid which was absorbed and determined the fraction of this that appeared in the thoracic duct lymph. We have also measured the changes in the composition of the portal and femoral blood that occurred simultaneously. As a control the effect of the administration of non-lipid material on the lipid content of blood and lymph was also studied.

Experimental. Well nourished young dogs weighing from 14 to 18 kgm. were used. From a week to ten days prior to the experiment a permanent cannula, as described by Horine (7), was placed on the portal vein, so that repeated samples of blood could be obtained without the

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[2] The data are taken from the thesis of J. Maxwell Little presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Vanderbilt University, 1941.

necessity of an open abdomen. The animals received their last food from 36 to 48 hours before the experiment was begun. They were anesthetized with either sodium barbital or Dial (Ciba) with urethane. An incision was made on the left side of the neck that exposed the external and internal jugular veins. The external vein was ligated distal to its junction with the internal jugular vein which was also ligated. The left subclavian and left innominate veins were ligated as well as several small veins emptying into the common jugular vein. In this way it was possible to cannulate the common jugular vein and collect lymph from the thoracic duct. In each case the course of the left thoracic duct and its several mouths, which were frequently found, was determined, so that the above ligations would not interfere with the flow of lymph.

A similar incision was made on the right side and all of the vessels entering the external and common jugular and innominate veins were ligated as well as the right thoracic duct. In this way entrance of lymph into the blood from the right duct was prevented, while maintaining an adequate venous return from the head.

All lymph flowing was collected for one hour and control samples of portal and femoral blood were drawn, after which the animal was given 100 cc. of milk or cream. To avoid the uncertainty due to the emptying time of the stomach, this was injected directly into the duodenum, exposed by a small incision made in the mid-line of the abdomen, which was immediately closed. The milk was skimmed milk which had been centrifuged to remove the residual fat and yield a fluid which was nearly lipid free but still similar to the cream in other respects.

Lymph was collected continuously throughout the experiment in tubes containing 0.1 cc. of a 3 per cent heparin solution. The collections were divided into arbitrary periods representing one hour intervals for the first two hours and two hour intervals thereafter. The volume of lymph for each period was recorded and aliquots were taken for extraction. Blood samples were drawn at the same time intervals and were immediately centrifuged and aliquots of plasma were extracted.

Throughout the experiment 50 to 100 cc. portions of normal saline were injected subcutaneously to replace approximately the volume of fluid withdrawn as lymph and blood. The experiment was terminated between the eighteenth and twenty-fourth hour after the injection of cream or milk. In some cases the flow of lymph ceased before the experiment ended, but in no case was this due to clotting. The flow of lymph was never stopped more than a few minutes by the rather infrequent clots which formed.

The animals were autopsied in each case to verify the location of the Horine cannula and to inspect the gastro-intestinal tract. In no case was there evidence of cream or milk in the lower part of the ileum. In

those animals receiving cream the stomach and small intestine were removed and thoroughly washed with several portions of distilled water. These washings were collected, and examined for lipids. In order to determine the efficiency of this recovery procedure, three experiments were performed in which 100 cc. of cream were injected into the duodenum. After ten minutes the animals were killed and the contents of the stomach and small intestines recovered and analyzed in the above manner.

These washings were evaporated to dryness in an oven at 80°C., and the dry weight of the residue was determined. After thorough mixing the lipid material from a weighed aliquot of the residue was extracted.

The procedures used for determining total and free cholesterol, lipid phosphorus, and total lipid carbon were, with some modifications, the manometric methods described by Kirk, Page and Van Slyke (9) and Van Slyke and Folch (17).

The lipids were extracted by 90 cc. of alcohol-ether at room temperature, which was found by Boyd (3) to be entirely satisfactory. Cholesterol esters were saponified by adding to the aliquot 0.18 cc. of a saturated aqueous solution of sodium hydroxide and 2 cc. of ethyl alcohol. Another modification was the substitution of the procedure of Man (11) for the evaporation of the alcohol-ether extract prior to extraction with petroleum ether.

The values obtained by these methods were converted into the more conventional terms by the calculations given by Page et al. (13), with the exception of true free cholesterol which was obtained by multiplying the observed free cholesterol by 0.8 (6, 16).

RESULTS. *Lymph:* A change in the concentration in lymph may be caused by a change in the quantity of the compounds reaching the lymph from the intestine, or by a change in the amount of fluid which reaches the lymph from the intestine or from contributory lymphatics. Since there are two variables which determine concentration of thoracic duct lymph, it is hazardous to draw conclusions from concentrations. We have accordingly used the expression quantity per hour rather than quantity per volume as a basis for comparison.

In table 1 are recorded the quantities of each lipid fraction for each period of collection. As would have been anticipated, the amount of total lipids and neutral fat in the lymph is greatly increased by intestinal absorption of lipids. In the two animals receiving milk, however, there was a decrease in the rate of total lipid and neutral fat transport.

In table 2 are found the data required for calculating the percentage of administered lipids that were absorbed and recovered in the lymph. We were able to recover an average of 93 per cent of the lipid injected into the duodenum in the control experiments, and the figures given in the third column have been corrected accordingly. In the case of dog 1 the absorp-

tion was not quite 70 per cent, while in the other two it was well above this. The figures in the fifth column are slightly too high, because the lipid which would have been present had no cream been given is included. Only a

TABLE 1

Lipid transport by lymph after injection of cream or milk

Results in milligrams

HOUR	SAMPLE	TOTAL CHOLESTEROL					FREE CHOLESTEROL					ESTERIFIED CHOLESTEROL				
		Cream			Milk		Cream			Milk		Cream			Milk	
		D1	D2	D3	D4	D5	D1	D2	D3	D4	D5	D1	D2	D3	D4	D5
0	L_0	22	5	11	6	78	5	1	4	2	21	17	4	7	4	57
0–1	L_1	25	6	13	6	55	6	2	2	2	16	19	4	11	4	39
1–2	L_2	17	16	9	*	80	4	5	2	*	25	13	11	7	*	55
2–4	L_3	28	33	11	6	54	9	14	5	2	16	19	19	6	4	38
4–6	L_4	21	33	14	4	43	7	16	7	1	12	14	17	7	3	31
6–8	L_5	18	35	6	7	36	6	13	4	3	11	12	22	2	4	25
8–10	L_6	18	27	11	6	25	6	10	1	2	7	12	17	10	4	18
10–12	L_7	22	38	2	5	14	7	12	0	2	5	15	26	2	3	9
12–14	L_8	21	35	1	5		7	9	0	2		14	26	1	3	
14–16	L_9	17	25		4		6	6		1		11	19		3	
16–18	L_{10}	17	21		4		6	5		2		11	16		2	
18–20	L_{11}	21	17		4		9	4		2		12	13		2	
20–22	L_{12}	12					5					7				
22–23	L_{13}	15					6					9				

HOUR	SAMPLE	PHOSPHATIDE					NEUTRAL FAT					TOTAL LIPIDS				
0	L_0	14	3	4	12	27	45	4	16	9	38	92	15	37	29	182
0–1	L_1	20	2	0	10	61	78	4	10	13	45	136	16	30	32	187
1–2	L_2	12	15	0	*	63	46	24	11	*	95	83	62	25	*	277
2–4	L_3	25	46	18	10	60	104	224	127	11	75	170	317	160	30	215
4–6	L_4	21	71	7	7	43	161	399	262	10	59	224	517	287	24	166
6–8	L_5	25	61	0	10	24	166	199	157	13	43	219	310	164	34	120
8–10	L_6	23	32	0	8	22	147	99	15	13	20	196	169	32	30	79
10–12	L_7	30	39	0	9	13	186	52	13	9	19	248	148	17	25	54
12–14	L_8	29	23	0	9		182	28	2	10		242	104	4	26	
14–16	L_9	15	9		8		93	10		12		132	58		25	
16–18	L_{10}	12	4		9		75	7		9		111	44		24	
18–20	L_{11}	29	4		8		221	6		12		278	36		24	
20–22	L_{12}	17					126					158				
22–23	L_{13}	20					158					198				

* L_2 combined with L_3 and represents lymph flowing during 3 hours.

small fraction (4–17 per cent) of absorbed lipids is transported by the left thoracic duct lymph during and immediately after the period of absorption.

Instead of a greater percentage transportation of lipids by the left thoracic duct, as might have been expected if absorption were incomplete

in the experiments of Munk and Rosenstein (12) and Eckstein (5), our results were considerably lower than those of the former authors and very similar to those of Eckstein. This deviation from the results of Munk and Rosenstein might be explained by the fact that their fasting period was much shorter than ours; it must also be remembered that they were using as subject a person with a definite pathological condition—elephantiasis.

The control animals showed a decrease in lymph phosphatide which was completely or partly overcome when cream was given. This increase was not related to the small amount given (80–190 mgm.) but was related to the rate of lipid absorption. This may be construed as evidence that phosphatide is synthesized during absorption of lipid. In previous reports it has been assumed that an increase in the concentration of phosphatides in thoracic lymph is evidence of synthesis. For reasons given above, mere changes in concentration are unreliable indications of changes in quantity.

Changes in the cholesterol fractions of lymph were not significant.

TABLE 2

Per cent of absorbed lipids recovered in lymph

DOG NUMBER	LIPID INJECTED AS CREAM	LIPID RECOVERED FROM INTESTINE	LIPID ABSORBED	LIPID RECOVERED IN LYMPH	PER CENT OF ABSORBED LIPID RECOVERED
	mgm.	*mgm.*	*mgm.*	*mgm.*	
1	21065	6999	14066	2395	17
2	20659	564	20095	1781	9
3	17739	1641	16098	716	4

Plasma: To facilitate the analysis of the data obtained for plasma the percentage change from the concentration found in the samples drawn in the control period were calculated for each lipid fraction for each period and for each dog. These percentages were then averaged to obtain a composite for each lipid fraction in blood from each of the two sources and for each type of experiment—injection of cream or milk. These data are presented in tables 3 and 4.

It has generally been assumed that if the entrance of thoracic duct lymph into the blood is prevented by ligation or by cannulation of the duct, any absolute increase in lipid concentration in the portal blood, or any relative increase in the portal when compared with some other blood, is evidence of direct blood transport. This assumption is hazardous because in the first place it has been shown (2, 10) that ligation of the duct frequently results in the establishment of collateral lymphatic circulation with consequent entrance of lymph into the blood by other channels, and in the second place because no attempts have been made to determine what effect the digestion and absorption of non-lipid materials had upon the blood lipid concentration.

We have found definite increases in the concentration of total lipids, neutral fat, phosphatide and free cholesterol (with a corresponding decrease in the bound form) in both the portal and femoral plasmas during

TABLE 3

Average per cent changes in plasma concentration above and below zero hour concentrations after administration of cream

P = portal, F = femoral

HOUR	TOTAL CHOLESTEROL		FREE CHOLESTEROL		ESTERIFIED CHOLESTEROL		PHOSPHATIDE		NEUTRAL FAT		TOTAL LIPIDS	
	P	F	P	F	P	F	P	F	P	F	P	F
1	−6.0	−2.0	+3.0	+3.0	−11.0	−4.0	−4.0	−12.0	+28.0	−3.0	0	−7.5
2	−3.0	−2.0	−2.0	−0.5	−3.0	−3.0	+15.0	−7.0	+7.0	+5.0	+2.0	−5.0
4	0	−1.0	+12.0	+11.0	−5.0	−6.0	+31.0	0	+30.0	+28.0	+13.0	−1.0
6	+2.0	0	+18.0	+15.0	−6.0	−7.0	+28.0	+1.0	+34.0	+13.0	+14.0	+2.0
8	+3.0	+3.0	+19.0	+25.0	−4.0	−7.0	+23.0	−1.0	+35.0	+40.0	+14.0	+7.5
10	+10.0	+6.0	+22.0	+22.0	+4.0	−1.0	+19.0	+5.0	+34.0	+44.0	+16.0	+12.0
12	+3.0	+1.0	+30.0	+26.0	−7.0	−12.0	+22.0	+5.0	+40.0	+41.0	+13.0	+10.0
14	+9.0	+7.0	+24.0	+26.0	+2.5	−2.0	+30.0	+8.0	+30.0	+26.0	+17.0	+9.0
16	+4.0	−2.0	+21.0	+24.0	−3.0	−15.0	+17.0	−4.0	+17.0	+57.5	+8.0	+10.0
18	+8.0	0	+40.0	+24.0	−6.0	−9.0	+52.0	−18.0	+36.0	+35.0	+21.0	−2.0
20	−2.0	−4.0	+16.0	+14.0	−9.0	−11.0	−11.0	−15.0	+16.0	+22.0	−3.0	−7.0

TABLE 4

Average per cent changes in plasma concentrations above and below zero hour concentrations after administration of milk

P = portal, F = femoral

HOUR	TOTAL CHOLESTEROL		FREE CHOLESTEROL		ESTERIFIED CHOLESTEROL		PHOSPHATIDE		NEUTRAL FAT		TOTAL LIPIDS	
	P	F	P	F	P	F	P	F	P	F	P	F
1	−5.0	+1.5	+4.0	+2.0	−9.0	+1.5	−9.0	−1.5	+30.0	+7.0	−0.5	+1.5
2	−1.5	+5.0	−7.5	−3.5	+1.0	+10.0	+20.0	+10.0	−7.5	+8.0	+2.5	+7.5
4	+1.0	+6.0	+4.0	+6.0	0	+5.5	−11.0	−2.0	+57.0	+6.0	+6.0	+3.0
6	+1.0	+5.0	+3.5	+2.0	+0.5	+6.5	+25.0	−13.0	+126.0	+0.5	+32.0	−3.0
8	+5.0	+11.5	+11.5	0	+2.0	+17.0	+30.0	−21.0	+5.0	−14.0	+12.5	−6.5
10	+3.5	+0.5	+13.0	+4.0	0	−1.5	+39.0	−0.5	+2.0	+6.0	+13.5	+2.0
12	−0.5	+11.5	+14.0	+1.5	−8.5	+17.0	+29.0	−14.0	+25.0	−9.0	+10.0	−3.0
14	+1.5	+9.5	+20.5	+19.0	−7.0	+3.5	+23.0	+4.5	+61.0	+40.0	+14.5	+15.0
16	−3.0	+1.0	+33.5	+18.5	−22.0	−9.0	+10.0	+4.0	+52.5	+19.0	+12.0	+12.5
18	−9.5	+6.5	+11.5	+25.0	−28.5	−5.0	+22.0	−18.0	+9.0	+7.0	+10.5	−4.5

the absorption of lipids. In most cases the rate of increase was greater in the portal than in the femoral. If these were the only data available, one would conclude that lipids are transported directly by the portal vein during absorption. However, since almost identically the same increases

in portal lipid concentration were found in the control animals which were absorbing non-lipid material, it must be concluded that changes in portal lipid concentration are not necessarily related to the absorption of lipid *per se* and cannot be considered as evidence for the direct transportation of absorbed lipids.

The explanation may be that there is a mobilization of lipids from depots, perhaps in the intestinal wall or mesentery. The stimulus for this mobilization must be the absorption process itself quite independent of the specific absorption of lipids. It is unlikely that experimental procedures are responsible since Eckstein (4) found that experimental manipulation and anesthesia alone produced no lipemia in femoral blood. It is likely that the so-called alimentary lipemia is due to two factors: *a*, entrance into the general circulation of absorbed lipids which are transported by the lymphatic system, and *b*, the mobilization of lipids by the portal vein from depots located in its area of drainage.

On the other hand, there is evidence that neutral fat and phosphatides do reach the general circulation during lipid absorption. It is probable that this is due to the presence of lymphatico-venous communications other than the well recognized one between the thoracic duct and the neck veins. This is a factor which has been almost universally neglected in studies of lipid absorption. Schmidt-Mülheim (14), Baum (1), Silvester (15), and Job (8) have described such communications.

It is of some interest that in the case of neutral fat, and for that reason also in the case of total lipid, there is a marked and rapid increase in femoral plasma concentration which appears late in the experiment. This is probably due to a delayed mobilization of neutral fat in the general circulation and appears to be a terminal phenomenon. This would explain the rather prolonged elevation in plasma concentrations found in the animals absorbing lipid.

SUMMARY

In three dogs the percentage of absorbed lipids which was transported by the left thoracic duct lymph during and immediately after absorption of lipids varied from 4 to 17 per cent. The rate of transport of total lipids, neutral fat and phosphatides increased during absorption.

There is a greater increase in the proportion of cholesterol in the free state in the general circulation during the absorption of lipids than during the absorption of non-lipids. There is a definite increase in the neutral fat concentration in the general circulation during the absorption of lipids and little change during the absorption of non-lipid material. There is little change in the concentration of phosphatide in the general circulation during the absorption of lipids, but there is a marked decrease during the absorption of non-lipids. These results are evidence that neutral fat and

phosphatide enter the general circulation during lipid absorption, probably by way of lymphatico-venous communications.

There is no evidence that absorbed lipid enters the portal blood directly. The increase in portal plasma lipids during non-lipid, as well as lipid absorption, suggests a mobilization of stored lipids by the portal blood under the stimulus of absorption itself. This phenomenon is being investigated further.

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THE EFFECT OF ACIDOSIS UPON THE RENAL TUBULAR REABSORPTION OF PHOSPHATE

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In a previous report (1) we demonstrated that the rate of reabsorption of phosphate by the renal tubules is an important factor in the regulation of the concentration of phosphate in the blood plasma at equilibrium. Vitamin D was found to increase the rate of renal tubular reabsorption of phosphate, thereby increasing the concentration of this ion in the plasma and other extracellular fluids. On the other hand, parathyroid extract decreased the rate of reabsorption of phosphate by the renal tubules and lowered the concentration of phosphate in the plasma. It has been repeatedly observed that the excretion of phosphate in the urine is increased in states of acidosis (2). Following the administration of acidifying salts to animals or man the increase in urine phosphate is not associated with an increased concentration of phosphate in the plasma but rather with a decrease in the plasma phosphate. In recent years cases of rickets not due to vitamin D deficiency have been reported in which a low concentration of phosphate in the serum was found associated with a severe chronic acidosis (4, 5, 6). It seemed probable that the rate of reabsorption of phosphate by the renal tubules was reduced in states of acidosis. The present experiments were designed to test this hypothesis.

The experiments were performed on dogs in whom an acidosis was produced by the oral administration of ammonium chloride. The degree of acidosis was measured by the reduction in serum bicarbonate as well as by determination of plasma pH. The rate of tubular reabsorption of phosphate was estimated by simultaneous determinations of the plasma creatinine and phosphate clearances following the intravenous injection of sodium phosphate. Since, in the dog, the plasma creatinine clearance is a measure of the filtration rate the difference between the creatinine and phosphate clearances can be used to calculate the amount of phosphate which has been reabsorbed by the renal tubules. The detailed procedure has been previously described (1).

When ammonium chloride is given in sufficiently large amounts to produce an acidosis a considerable loss of extracellular fluid volume and hemo-

concentration may result and consequently a reduction in the rate of glomerular filtration as measured by the creatinine clearance is found. It was therefore first necessary to study the effect of variations in the rate of glomerular filtration upon the tubular reabsorption of phosphate. In many of the experiments in which spontaneous fluctuations in the rate of glomerular filtration occurred during successive periods, the rate of tubular reabsorption appeared to vary with the filtration rate. These findings were not entirely constant and the effect of marked variations in the rate of glomerular filtration was therefore determined. This was accomplished by decreasing or increasing the extracellular fluid volume sufficiently to produce a significant decrease or increase in the filtration rate. A decreased extracellular fluid volume was produced by the intraperitoneal injection of 5 per cent glucose and removal 5 hours later of the peritoneal fluid according to the technique of Darrow and Yannet (7). A mild degree of dehydration was also produced in one animal by the injection of a mercurial diuretic, salyrgan. Increase of the filtration rate was brought about by the continuous intravenous injection of a solution of sodium chloride and sodium bicarbonate of approximately the composition of extracellular fluid. The rate of injection was varied from 5 to 10 cc. per minute for a period of 2 hours.

TABLE 1

Effect of dehydration upon glomerular filtration and renal tubular reabsorption of phosphate

TREATMENT	URINE VOLUME	CREATININE CLEARANCE	PHOSPHATE REABSORBED	
			Mgm. per min.	Mgm. per 100 cc. glom. filtrate
Dog B				
	cc. per min.	*cc. per min.*		
Control	1.3	50.5	2.24	4.4
5 per cent glucose I.P.	1.2	34.5	1.47	4.3
Dog A				
Control	1.4	32.8	1.04	3.2
Control	1.8	33.1	1.07	3.2
Salyrgan	2.1	27.1	0.93	3.4
5 per cent glucose I.P.	0.5	8.8	0.36	4.1
Control	1.8	33.3	1.18	3.5

The results of these experiments are shown in tables 1 and 2. In the experiments in which dehydration had been produced sufficient water was given by stomach tube at the time of the experiment to maintain an ade-

quate flow of urine. The rate of reabsorption of phosphate is expressed both as milligrams phosphorus per minute and as milligrams phosphorus per 100 cc. of glomerular filtrate. When the rate of glomerular filtration, as measured by the creatinine clearance, is reduced there is seen to be an approximately proportionate reduction in the rate of reabsorption of phosphate by the renal tubules. The amount of phosphate reabsorbed in terms of volume of glomerular filtrate therefore remains fairly constant. However, when the rate of filtration is increased above the normal by a continuous intravenous injection, the tubular reabsorption of phosphate reaches a maximum which is not increased by further increase in the filtration rate. The equilibrium concentration of phosphate, i.e., the concentration of phosphate in the plasma at which the amount filtered through the glomeruli equals the amount reabsorbed by the renal tubules is therefore reduced by increasing the rate of glomerular filtration. Shannon and Fisher (8) have shown that in the normal dog the tubular reabsorption of glucose reaches a maximum which remains constant despite changes in the rate of filtration.

TABLE 2

The effect of increased glomerular filtration upon rate of renal tubular reabsorption of phosphate

CREATININE CLEARANCE	URINE VOLUME	PHOSPHATE REABSORBED	
		Mgm. per min.	Mgm. per 100 cc. glom. filtrate
Dog C			
cc. per min.	*cc. per min.*		
62.3	0.5	3.39	5.44
65.5	0.6	3.51	5.36
Constant intravenous injection of saline bicarbonate solution			
71.3	0.6	3.54	4.97
75.3	0.4	3.68	4.89
81.2	1.2	3.74	4.61
88.5	1.9	3.76	4.25
Dog D			
41.6	0.4	1.24	2.98
39.3	0.3	1.14	2.90
Constant intravenous injection of saline bicarbonate solution			
42.0	0.4	1.20	2.86
44.9	0.6	1.11	2.47
48.4	1.3	1.09	2.25
53.4	2.5	1.12	2.10
57.7	4.6	1.10	1.96

In their experiments a constant intravenous infusion of glucose solution was given and the conditions are therefore comparable to those experiments in which saline was injected. In the dehydrated dog the decrease in the rate of tubular reabsorption of phosphate in proportion to the decrease in filtration rate suggests that many of the glomeruli are not functioning and therefore no phosphate is available for reabsorption by the tubules connected with them. However, this phenomenon of glomerular intermittence which has been observed in the amphibian (9) has not been demonstrated in the normal dog by the injection technique (10). No studies by this method have been made in the dehydrated dog. Whatever the explanation may be it is apparent that in states of dehydration the reduction in filtration rate is associated with a comparable reduction in the estimated rate of phosphate reabsorption. For purposes of comparison it is preferable to express the reabsorption of phosphate in these experiments in terms of milligrams phosphorus per 100 cc. of glomerular filtrate.

TABLE 3

The effect of acidosis on renal tubular reabsorption of phosphate

Dog A

DATE	TREATMENT	CREATININE CLEARANCE	PHOSPHATE REABSORBED		SERUM	
			Mgm. per min.	Mgm. per 100 cc. glom. filtrate	P	CO_2
		cc. per min.			*Mgm. per 100 cc.*	*volume per cent*
12/29	Control	30.8	1.28	4.2	5.6	50.0
1/2–1/4	NH_4Cl—5 grams daily					
1/5		24.8	0.90	3.7	3.2	29.0
1/8	Recovery				4.0	50.7
1/21–1/23	NH_4Cl—7 grams daily					
1/24		26.5	0.69	2.6	3.0	16.6
2/27	Control	29.9	1.25	4.2	4.6	
3/2–3/4	NH_4Cl—5 grams daily					
3/5		29.3	0.95	3.2	4.1	30.4

The results of the experiments in which ammonium chloride was given are shown in tables 3, 4 and 5. Control studies were done both before and after the acidosis experiments and the experimental prodecure was identical in all cases. The plasma pH was determined in many of the experiments, using a Leeds and Northrup Universal pH indicator employing a glass electrode which permitted the determination without exposure of the sample to air. A reduction of plasma pH was found consistently following the administration of ammonium chloride in the amounts given and the reduc-

TABLE 4

The effect of acidosis on renal tubular reabsorption of phosphate

Dog B

DATE	TREATMENT	CREATININE CLEARANCE	PHOSPHATE REABSORBED		SERUM	
			Mgm. per min.	Mgm. per 100 cc. glom. filtrate	P	CO_2
		cc. per min.			*Mgm. per 100 cc.*	*volume per cen*
12/3	Control	57.2	3.73	6.5	7.2	
12/6–12/7	NH_4Cl—10 grams					
12/8	NH_4Cl—5 grams					
12/9	Vomited NH_4Cl given					
12/10		47.2	2.22	4.7	4.6	38.7
12/17	Recovery	50.8	3.05	6.0	6.5	60.0
1/2–1/6	NH_4Cl—5 grams daily					
1/7		42.8	2.51	5.8	6.4	45.0
1/12–1/15	NH_4Cl—10 grams daily					
1/16		47.0	2.28	4.9	5.1	36.2
1/21	Recovery	45.6	2.57	5.6	6.4	62.4

TABLE 5

The effect of acidosis on renal tubular reabsorption of phosphate

Dog C

DATE	TREATMENT	CREATININE CLEARANCE	PHOSPHATE REABSORBED		SERUM	
			Mgm. per min.	Mgm. per 100 cc. glom. filtrate	P	CO_2
		cc. per min.			*Mgm. per 100 cc.*	*volume per cent*
4/10	Control	51.8	3.63	7.0	7.3	47.2
4/15	NH_4Cl—10 grams					
4/16	NH_4Cl—7.5 grams					
4/17		47.8	2.34	4.9	5.1	19.2
4/18	Recovery				6.3	38.1
4/22	Recovery	56.7	3.32	5.9	6.1	48.2
5/13	Control	63.9	3.45	5.4	5.5	
5/23–5/29	NH_4Cl—6 grams daily					
5/29		60.7	2.54	4.2	4.0	28.5
5/30–6/3	NH_4Cl—6 grams daily					
6/2					3.1	21.3
6/3		43.4	1.43	3.3	2.8	28.0
6/11	Recovery	67.7	2.20	3.3	3.9	46.7
6/17	Recovery	66.6	2.90	4.4	5.2	46.6

tion ranged from 0.11 to 0.27 pH unit. The degree of reduction in pH paralleled the decrease in serum bicarbonate.

In the tables are listed the plasma creatinine clearance and the calculated rate of tubular reabsorption of phosphate expressed both as milligrams phosphorus per minute and as milligrams phosphorus per 100 cc. of glomerular filtrate. The concentration of phosphate and of bicarbonate in the serum in the fasting state on the morning of the experiment is also given.

There is obviously a considerable decrease in the rate of tubular reabsorption of phosphate following the development of a severe acidosis. In those experiments in which the creatinine clearance is lowered the reduction in the rate of tubular reabsorption of phosphate is far greater than that expected as the result of the diminished filtration rate. This is shown by the fact that the reabsorption per volume of glomerular filtrate is decreased. The magnitude of the decrease in each animal is to some extent proportional to the severity of the acidosis. This is shown particularly by the results for dogs A and B in tables 4 and 5, respectively. The results for dog B are of particular interest. On 1/7 despite the administration of 5 grams NH_4Cl for 4 days there is only a mild degree of acidosis and no significant reduction in the rate of tubular reabsorption of phosphate is found. The administration of NH_4Cl was discontinued and the animal permitted to recover. A second course of NH_4Cl treatment was followed by a more severe acidosis. At this time 1/16, a definite decrease in the rate of renal tubular reabsorption of phosphate, is found.

The decrease in tubular reabsorption of phosphate appears to be related not only to the severity of the acidosis but also to the duration. In the experiments on dog C a moderate degree of acidosis was produced on 5/29 and the rate of tubular reabsorption of phosphate determined. The administration of NH_4Cl was continued in amounts sufficient to keep the serum bicarbonate at the same low level and it is seen that after a total of 11 days of NH_4Cl treatment there is a much greater decrease in the rate of tubular reabsorption which is not due merely to the greater reduction in the filtration rate.

The recovery of renal tubular function following cessation of the NH_4Cl administration is also an interesting phenomenon. In table 3 the results of the experiment on dog A indicate a complete return to the control values following the cessation of ammonium chloride treatment. This was an adult animal in whom the rate of tubular reabsorption of phosphate is fairly constant under normal conditions. In the case of dogs B and C the rate of tubular reabsorption of phosphate in the recovery experiments does not return completely to the preacidosis level. This probably is explained by the fact that these were animals less than 18 months of age and at this period a gradual decrease in the rate of tubular reabsorption of phosphate is normally observed (1). The recovery process is seen to be a rapid one

in those experiments in which an acidosis of short duration was produced. However, in the experiment on dog C shown in table 5 in which the acidosis was more prolonged it may be seen that the rate of tubular reabsorption of phosphate remained low for at least one week following cessation of NH_4Cl treatment even though the serum bicarbonate returned to normal. After 14 days, however, a definite increase in the rate of tubular reabsorption of phosphate is found although still below the control level. In another experiment on dog B there was also suggestive evidence that the renal tubular reabsorption of phosphate may return only slowly to the normal level even though the normal acid base equilibrium of the plasma has been restored. In this experiment the concentration of plasma phosphate remained low for several days after the bicarbonate had been restored to normal.

The concentration of phosphate in the plasma in the postabsorptive state is seen to parallel closely the rate of tubular reabsorption of phosphate as expressed in milligrams phosphorus per 100 cc. of glomerular filtrate. This is in accord with the previously expressed idea that the concentration of phosphate in the plasma tends to approach the "equilibrium concentration", i.e., the concentration at which the rate of filtration through the glomeruli equals the rate of reabsorption by the renal tubules (1). Numerically this equilibrium concentration is identical with the value of the phosphate reabsorbed per 100 cc. of glomerular filtrate.

The serum calcium was determined in all of the experiments. No consistent change in the concentration of serum calcium was noted in these studies.

DISCUSSION. The effect of acidifying salts upon the renal tubular reabsorption of phosphate is similar to that of parathyroid extract (1). Both cause a decrease in the rate of renal tubular reabsorption of phosphate and therefore a decreased concentration of phosphate in the plasma. The effect of acidosis differs from that due to hyperparathyroidism in that there is no concomitant elevation of the concentration of calcium in the serum. The results of the present experiments offer an explanation for the low concentration of phosphorus in the serum which may be found in states of chronic acidosis. The exact mechanism by which the acidosis affects the function of the renal tubules is still unknown.

The loss of phosphate from the body following the administration of acidifying salts is in excess of that which could come from the extracellular fluids of the body alone (3). There is direct evidence that this excess is derived from the intracellular organic phosphates since the acid soluble phosphate of the red blood cells is found to be decreased in states of acidosis (3,11). It is quite probable that there is an interrelationship between the effect of acidosis on renal tubular reabsorption of phosphate and the decrease of intracellular phosphate. The present study emphasizes the

fundamental importance of renal tubular function in maintaining the concentration of phosphate in the plasma and other body fluids at normal levels.

SUMMARY

The rate of reabsorption of phosphate by the renal tubules was determined in dogs in whom an acidosis was produced by the administration of ammonium chloride. It was found that following the development of an acidosis there was a decrease in the rate of tubular reabsorption of phosphate which was related both to the severity of the acidosis and its duration. The decreased concentration of phosphate in the plasma following the administration of acidifying salts can be explained by this effect on the renal tubular reabsorption of phosphate.

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THE PRESSOR RESPONSE OF NORMAL AND HYPERTENSIVE DOGS TO RENIN AND ANGIOTONIN

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Inconclusive evidence suggests that the blood vessels of hypertensive animals and man are abnormally sensitive to pressor agents, hence no more than normal amounts of them in the blood would be required to cause hypertension. It is, therefore, of importance to learn whether this is true of such substances as renin and angiotonin because of the belief that they may be involved in the genesis of chronic arterial hypertension. It is this problem with which this investigation is concerned.

Purified renin is not of itself a pressor agent and requires the intervention of renin-activator (1) before eliciting vasoconstriction by formation of angiotonin (2, 2a). In normal animals the supply of renin-activator is easily exhausted, but more of this substance appears available in the blood of hypertensive animals (3). It would, therefore, be anticipated that hypertensive animals would respond to injections of renin with a greater or, at least, more prolonged rise of arterial pressure than normal animals simply because more angiotonin is formed rather than that hypertensive animals are more sensitive to the action of angiotonin; hence increased pressor sensitivity of hypertensive animals might reflect an increased supply of renin-activator. An altered response of the vessels themselves to renal pressor agents can only be assumed if it can be shown to exist with reference to the effective pressor substance, angiotonin.

METHODS. Dogs of from 10 to 14 kgm. weight were anesthetized by intraperitoneal injections of 30 mgm. of pentobarbital per kilogram body weight. The femoral artery was cannulated and connected to a mercury manometer by a tube filled with heparin solution. Injections were made into the femoral vein.

Renin was prepared by the method of Helmer and Page (4), and angiotonin by that of Page and Helmer (5). Single injections of renin always consisted of 0.3 cc., and angiotonin 0.25 cc., the angiotonin being given before the renin. When renin was infused, it was diluted in the proportion of one part in ten of physiological saline solution and given at the rate of 0.8 cc. per minute. The infusion was discontinued after 45 minutes. The

animal often was retested a week later and then hypertension induced by wrapping one or both kidneys in silk (6). Usually within three weeks or more, hypertension of the order of 180 to 210 mm. Hg mean pressure had developed and the tests were repeated. Blood pressure was measured in these animals usually on alternate days by femoral intra-arterial puncture with a no. 20 needle.

Results. It was soon evident that the pressor response of the different normotensive dogs varied greatly, especially to renin and less so to angiotonin. The same animal tested on different occasions exhibited less variability. At first we thought this might be due to different depths of anesthesia. This is apparently not the case for, in 6 animals, increasing the dose of pentobarbital progressively had no regular effect on the pressor response to angiotonin. In several docile, well-trained animals tests were made without anesthesia and later compared with those made while the animal was anesthetized. Just as had been found before (7), no regular differences were encountered; however, since some of the animals became excited, especially at the height of the blood pressure rise, it was believed that experimentally comparable conditions could best be attained by the use of pentobarbital anesthesia. Furthermore, few dogs could be trained to lie quietly for 40 minutes during an infusion of renin.

It also seemed desirable to determine whether the level of blood pressure could consistently influence the results. Experience shows that at both extremes of blood pressure the response to *angiotonin* is reduced. When arterial pressure is elevated or depressed less extremely, there does not appear to be any direct relationship between the level of blood pressure and the pressor response. For example, the responses to angiotonin of one dog with arterial pressure at 240 mm. Hg were 18 and 14 mm. Hg, but one with arterial pressure of 174 also showed similar responses, namely, 20 and 14 mm. Hg. One dog with arterial pressure at 224 mm. Hg responded to angiotonin by rises of 36 and 32 mm. Hg while in another with blood pressure of 180 the responses were also 36 and 34 mm. Hg.

Page (7) was unable to demonstrate any deciding influence on the pressor response to *renin* of the initial level of blood pressure in normal animals. As table 1 shows, the same appears to be true of hypertensive animals. It makes no difference whether the initial blood pressure chosen is the *average* daily pressure determined without anesthesia or one measured under anesthesia, just before the renin is administered. For example, a rise of 48 mm. Hg occurred in an animal in which the average daily pressure was 224 mm. Hg and the initial pressure under anesthesia was 162 mm. Hg. Yet a rise of 50 mm. Hg occurred in another animal with an average daily pressure of 228 mm. Hg, but an initial pressure under anesthesia of 210 mm. Hg. It is possible that at extremes of pressure, such as 230 mm. Hg and 240 mm. Hg mean pressure, the response is reduced.

Having selected suitable conditions for testing the response of the animals to angiotonin and renin, 6 dogs were tested; in two cases (no. 13 and no. 8) twice, and hypertension induced by silk perinephritis. When various levels of elevation of mean arterial pressure ranging from 140 to 224 mm. Hg were attained, the animals were again tested (table 2). While there was a slight increase in the response to angiotonin it was not consistent. The response to renin was slightly greater in all experiments.

It seemed desirable to ascertain the sensitivity in a different way. Instead of giving the renin by single injections, it was infused in dilute solution. Six animals were tested in this manner before and after the induction of hypertension (table 3). Again the same range of blood pressure levels

TABLE 1

Relationship of renin pressor response to arterial blood pressure in hypertensive dogs

DOG NUMBER	AVERAGE B.P. LEVEL OF UNANESTHETIZED DOG	B.P. BEFORE INJECTION, WHILE ANESTHETIZED	RISE AFTER RENIN
			mm. Hg.
1	174	190	22
2	180	210	22
3	180	160	28
4	180	184	28
5	182	214	34
6	194	170	26
7	198	204	30
8	202	226	28
9	210	200	50
10	212	186	54
11	212	210	20
12	220	220	22
13	224	162	48
14	228	210	50
15	230	230	20
16	240	240	16

was chosen. The response to angiotonin, just as in the last group of experiments, was slightly greater but not consistently greater in the hypertensive animals. With one exception (no. 20), the response to infused renin was both greater and more prolonged even on repeated infusions. These experiments thus support the results of the group in which the increased response of hypertensive animals to renin was shown by single injections.

It then seemed desirable to test a larger series of normal and hypertensive dogs with single injections of both angiotonin and renin, but in this group the same animals were not necessarily tested before and after the induction of hypertension. Amounts of each were used such that the rise in blood pressure was not excessive. As Swingle, Taylor, Collings and Hays (8)

have shown, when small doses of renin are employed the initial blood pressure does not effect the pressor response elicited. Fifty-seven normotensive animals were tested with angiotonin and thirty-six hypertensive, the blood pressure of the latter all being over 170 and averaging 200 mm. Hg.

It was necessary to use such a large number of animals because the variability in response from one animal to another was great. In table 4 the normal and hypertensive animals are grouped together according to the particular sample of angiotonin used for test purposes. The results show

TABLE 2

Pressor response of dogs to renin and angiotonin before and after induction of perinephric hypertension

NORMAL DOGS				HYPERTENSIVE DOGS		
Dog number	Initial B.P.	Rise of B.P. after angiotonin	Rise of B.P. after renin	Average B.P. after induction of hypertension	Rise of B.P. after angiotonin	Rise of B.P. after renin
	mm. Hg					
17	122	14	22	140	24 24	28
16	134	10 12	16	160	28 30	28
18	130	30 28	10	174	20 14	22
19	120		20	198	30 18	30
8	116	26	20 20	202	22 18	28
13	138 144	22 24	32 32	224	36 32	48

that there is no significant difference in the response to angiotonin in normo- and hypertensive dogs.

Twenty-four normo- and hypertensive animals were tested with single injections of renin. The results on inspection show a small but consistent increase in sensitivity, which, however, is not statistically significant.

DISCUSSION. The evidence concerning the sensitivity of the vascular system to pressor drugs in hypertension is conflicting. For this reason it is necessary to consider in some detail certain questions concerning the technique of the measurements of sensitivity and their interpretation.

Many of the conflicting reports are due in no small part to the inadequate

TABLE 3

Pressor response to single injections of angiotonin and infusions of renin before and after induction of hypertension

DOG NUMBER	INITIAL B.P.	RISE OF B.P. AFTER ANGIOTONIN	RESPONSE TO RENIN INFUSION: Under anesthesia: Initial B.P.	10	20	30	40 minutes
Before induction of hypertension							
	mm. Hg.						
20	132	18 14	172	18	18	4	0
21	122	18 20	136	32	40	34	24
22	130	14 14	204	16	0	−14	−22
23	120	16 14	180	14	6	2	0
24	134	20 22	148	42	32	20	16
25	118	28 20	164	36	36	14	−4
After induction of hypertension							
	AVERAGE B.P. AFTER HYPER-TENSION						
20	180	8 10	266	6	−16	−36	−56
20	160	8 10	246	12	−22	−32	−40
21	176	16 16	190	44	44	38	26
21		16 16	182	36	28	12	8*
22	196	18 20	220	44	30	12	2
22	198	22 20	180	40	54	36	26
23	200	28 22	148	52	66	58	52
24	210	24 22	192	54	30	22	32†
25	192	36 34	196	48	44	44	30

* Malignant.

† Early malignant.

number of animals employed in the experiments. Experience shows that the variation from one animal to another is great, and even in the same animal sufficient changes in sensitivity occur to make exact comparison difficult. Experiments in which the same animal is tested before and after induction of hypertension therefore seem most reliable. The cause of this great variability is not known and deserves further study.

The use of pentobarbital anesthesia appears to be the lesser of two evils because it has been shown that in moderate amounts it does not greatly alter the intensity of the pressor response to renin and angiotonin, and even varying the depth of the anesthesia does not consistently alter the response one way or another.

The problem whether the response to pressor agents in general is directly dependent on the height of the arterial blood pressure is no simple one, even

TABLE 4

*Pressor response to single doses of angiotonin and renin compared in normal and hypertensive dogs**

LOT NUMBER	MEAN ± S.E. IN NORMAL DOGS	MEAN ± S.E. IN HYPERTENSIVE DOGS	VALUE OF t	D/F	VALUE OF t NECESSARY TO SHOW SIGNIFICANCE		SIGNIFICANT
					19/20	99/100	
Angiotonin 647	19.38 ±1.144	29.00 ±0.577	7.51	15	2.13	2.95	Definitely
Angiotonin 751	16.04 ±0.779	23.50 ±2.875	2.50	38	2.02	2.70	Barely
Angiotonin 226	26.95 ±2.325	22.67 ±2.658	1.21	50	2.01	2.68	No
Angiotonin 923	18.86 ±2.342	29.43 ±2.296	3.22	12	2.18	3.06	Yes
Renin 725	23.46 ±2.075	28.12 ±3.009	1.27	42	2.02	2.70	No

* We are grateful to Mr. E. B. Robbins and Dr. K. K. Chen for the statistical analysis.

when adrenalin is employed. The size of the dose, the rate of administration, the temperature of the animal and the intactness of the nervous system are all factors known to influence the response (9). When the blood pressure is lowered it depends on the means employed to lower it (10), and when adrenalin is injected repeatedly, the response depends on the number of injections (11–13).

The response to renin and angiotonin is not directly related to the initial height of the blood pressure. Immediately after pithing, it may be completely abolished and that to angiotonin greatly reduced. After several injections of angiotonin the response increases. Severe hemorrhage reduces the response, especially when the arterial pressure is markedly lowered. The method employed to lower pressure seems to determine whether an increased or decreased response will occur.

Some light is thrown by the results in table 1 on the question whether

arterial pressure measurements taken while animals are anesthetized correspond with those taken without anesthesia. It is clear that the variations of the blood pressure taken under pentobarbital anesthesia from the usual daily pressure taken by femoral arterial puncture without anesthesia is great in many cases. It is true that the pressures often correspond but not with sufficient regularity to justify use of the method except where large groups of animals are employed.

We are now in a position to correlate the results of experiments in which the purpose was to ascertain whether hypertensive animals respond more actively to angiotonin and other pressor agents than do normotensives.

Verney and Vogt (14) studied the pressor response of 7 hypertensive dogs to adrenalin. In one a decreased response was observed, in 3 no change, and in 3 others an increase was noted.

Appelrot (15) claimed that 10 dogs with hypertension produced by *feeding large doses of vitamin D exhibited decreased rather than increased* pressor responses to adrenalin when compared with normotensive dogs. Since blood pressure measurements were made under ether anesthesia without controls in which no anesthesia was employed, and since the hypertension was for the most part very moderate (average rise of 32 mm. Hg in 10 dogs fed vitamin D, compared with a 9 mm. Hg rise in 6 normal dogs), it is doubtful how much weight can be given these experiments.

The same irregularity of response was observed by Verney and Vogt (14), when tyramine was injected in single doses. The range of rise in normal dogs was 26 to 85, and hypertensive dogs 50 to 98 mm. Hg. When tyramine was infused into 3 dogs the hypertensives responded much *less* actively than normal ones. These results, obtained on a small number of animals, suggest that the vascular system of hypertensive animals is not hypersensitive to tyramine. Verney and Vogt were also unable to confirm Bouckaert, Elaut and Heymans' (16) observation that the pressor response to carotid sinus stimulations of 3 hypertensive dogs under chloralosane anesthesia, was increased.

Brown and MacGraith (17, 18) measured the response to drugs of 12 rabbits made hypertensive by constricting the renal artery, and 4 with glomerulonephritis induced by nephrotoxic serum. Blood pressure measurements were made on Van Leersum loops while the animals were under pentobarbital anesthesia. The degree of hypertension was not great. The pressor response to adrenalin, tyramine and posterior pituitary extract was increased and the depressor response to acetylcholine decreased as early as 10 hours after operation. Once the hypertension was established, the response varied roughly as the blood pressure level. In a later study (19) it was sought to determine whether the possible increase of sensitivity to tyramine was due to loss of tyraminase activity, but it was found that

slices of liver of hypertensive rats oxidized tyramine at normal rates. Ogden, Brown and E. W. Page (20) independently observed increased sensitivity in 16 of 17 rabbits in the pre-hypertensive stage, i.e., after constricting the renal artery but before any appreciable rise in arterial pressure had occurred. Similar studies have not been made in dogs.

Several studies have been carried out in which renin has been the pressor agent. While the renin employed in most cases leaves much to be desired from the point of view of chemical purity, nevertheless the results show a fair degree of consistency. Thus Leiter and Eichelberger (21) state in an abstract of their work that 20 injections into 4 unanesthetized hypertensive dogs caused a more sustained rise of blood pressure than in control animals. Page and Helmer (5) compared the response of 9 normal and 2 hypertensive dogs to infusions of renin. The hypertensive dogs showed both a greater and more sustained rise in pressure. Katz and Friedberg's (23) results with single injections of renin show slightly greater pressor response to renin in 3 of 4 hypertensive dogs, but they interpret these results as indicating similar responses in normo- and hypertensive dogs.

Heated kidney extracts tested by Kapp, Friedland and Landis (24) caused a greater rise of arterial pressure in 3 unanesthetized hypertensive rabbits than in 3 normal ones. When more concentrated extracts were used and the initial blood pressure was higher, the results in 5 hypertensive rabbits became irregular. According to Williams, Wegria and Harrison (25), 4 hydronephrotic rats with hypertension showed an average rise of about 20 mm. Hg greater than 3 control rats when renin was injected.

The more extensive data reported in this paper makes it appear probable that hypertensive dogs are slightly more sensitive than normal to renin, especially when subjected to the continuous administration of renin. This we interpreted as being due to increased renin-activator content of the blood of hypertensive animals (3), with formation of greater amounts of angiotonin. To angiotonin itself there seems to be no greater than normal pressor response in hypertensive dogs.

These experiments lend no support to the belief that the vessels of hypertensives are abnormally sensitive to substances which may participate in the chemical mediation of hypertension. Since angiotonin and renin may be among these substances, this observation assumes some importance.

SUMMARY. 1. The pressor response to renin and angiotonin is not consistently altered by moderate pentobarbital anesthesia. The initial arterial pressure level of hypertensive dogs may be profoundly altered by anesthesia.

2. The pressor response of different animals varies widely, but that of the same animal is relatively constant even at different times.

3. The pressor response to angiotonin and renin is not directly related to the initial arterial pressure. The means employed to lower or raise the pressure appear to determine in large measure the response.

4. Induction of hypertension in dogs does not alter the pressor response to angiotonin but increases that to renin slightly, especially if the renin is administered by infusion.

CONCLUSION

Induction of experimental renal hypertension in dogs does not increase the pressor response to angiotonin. On the other hand, in such animals renin causes somewhat greater responses. The increased response to renin appears to be due to greater formation of angiotonin from combination of renin and renin-activator, and is not the result of increased sensitivity of the vascular system of hypertensive animals.

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THE EFFECT OF INSULIN ON CARDIAC AND LIVER GLYCOGEN

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Earlier observations (1) using subcutaneously injected insulin in rats fed glucose by mouth gave irregular effects on cardiac glycogen even though the deposition of gastrocnemius glycogen was marked in each animal. When the results for animals showing hypoglycemic signs and low terminal blood sugars were separated from the others, it appeared that hypoglycemia was accompanied by a lowering of cardiac glycogen, whereas in the animals in which hypoglycemia apparently did not occur, the cardiac glycogen was raised as compared either to 24 hour fasted controls or to such controls given glucose by mouth. To clarify these irregular results, the observations recorded in this paper, and employing intravenously injected glucose and insulin, were made.

The action of insulin on liver glycogen has been difficult to reconcile with its other known actions (2, 3, 4, 5). The marked raising of the low liver glycogen of diabetic animals by insulin injection seemed to be a potent argument in favor of its effect being like that seen for muscle glycogen; but it has been recognized for normal animals that insulin given with glucose resulted in a lower liver glycogen than would have occurred if glucose alone had been given. Because the experimental arrangements for observing cardiac glycogen could also conveniently include observations on liver, the two were made together, it being felt for reasons which will be mentioned in discussion that the insulin effect on liver glycogen could stand re-observation.

Experimental. Male rats, 24-hour fasted, weighing between 150 to 200 grams were lightly anesthetized with nembutal and given glucose by constant intravenous injection at the rate of 500 mgm. per 100 grams of rat per hour; to this injected fluid in some of the experiments insulin was added. The urine was collected and analyzed for glucose. At the termination of the injection period, a gastrocnemius, the heart, liver and a sample of blood were obtained. Analyses were carried out as previously described (1, 6, 7). The adrenalectomized animals were maintained on 1 per cent sodium chloride solution as drinking water and used on the third day post-operatively. The results are given in the table.

Discussion. *Heart.* Intravenous glucose alone effected a definite

increase of cardiac glycogen. This was rather surprising in view of the earlier observation that glucose by mouth did not raise cardiac glycogen; indeed, the cardiac glycogen both of normally fed animals and also of 24-hour fasted animals absorbing glucose maximally for 4 hours was significantly lower than that of 24 or 48-hour fasted animals (1). In the present experiments the insulin is seen not to effect any further increase in cardiac glycogen. It would appear, therefore, either that the animals' own insulin acts maximally or that insulin is not necessary for the deposi-

TABLE 1

Effect of glucose and insulin intravenously

	Group	Number of animals	Hours injected	Per 100 grams body weight			Final concentration—carbohydrate mgm. per cent				Mgm./100 body weight			
				Injected		Retained	Glycogen			Glucose	Increase in Glycogen			Carbohydrate not accounted for
				Glucose	Insulin units	Glucose	Gastrocnemius	Heart	Liver	Blood	Liver	Other	Total	
				mgm.		*mgm.*								
Intact	1	16					521	542	192	87				
							±8*	±17	±32	±5				
	2	13	2	500		880	750	657	1761	329	78	114	192	567
							±14	±23	±141	±14	±7†	±11		
	3	4	2	500	¼	941	877	648	1038	222	42	178	220	654
	4	4	2	500	½	959	846	687	824	204	31	162	193	708
	5	11	2	500	1	946	884	628	277	252	4	181	185	679
							±26	±19	±56	±16	±3	±14		
	6	5	2	500	2–5	974	900	694	141	434	−3	186	183	615
	7	4	2	500		1440	980	646	472	383	14	229	243	(1049)
			+1	500	1									
Adrex	8	12					462	387	136	58				
							±13	±41	±8	±4				
	9	4	2	500		904	553	428	1174	466	52	46	98	602
	10	4	2	500	1	936	690	457	159	382	1	114	115	659

* Standard deviation of mean.
† Standard error of difference.

tion of cardiac glycogen, this last interpretation being supported by the phenomenally high cardiac glycogen of diabetic animals (8), which in the light of these experiments may not be due simply to hyperglycemia.

The cardiac glycogen of adrenalectomized animals is lower than that of controls; the increases produced by intravenous glucose over fasted controls and by glucose and insulin, as compared to glucose alone, were not large; the difference between fasted controls and animals injected with glucose and insulin was quite definite.

Liver. The derived values for carcass glycogen and for total glucose have been obtained by assuming the values for gastrocnemius glycogen and for blood glucose, respectively, to be distributed through 50 per cent of body weight. The values for carbohydrate unaccounted for is then the difference between glucose retained and the sum of the increases in glycogen and glucose. These assumptions are only approximately valid but are justifiable on a comparative basis in as far as they make for more quantitative interpretation of the data obtained. It is probable that they are as valid as would be respiratory quotients obtained under these conditions.

Considering groups 2 to 6, inclusive, the striking finding is the progressive decrease in liver glycogen as the insulin dose is increased. This effect of insulin on liver glycogen has been repeatedly observed. It is commented upon in the reviews of Cori (2), Best (3) and Soskin (5). The work of Bridge (9) has recently re-emphasized it. Others who have called attention to it include Cori and Cori (10, 11), Reid (12), Bodo and Neuwirth (13), Lundsgaard (14) and Russell (15). It is again recorded in this paper because a few new experimental circumstances are attached to the primary observation. A number of objections to admitting the evidence at its face value have been or can be made and will be briefly discussed.

The phenomenon would not appear to be dependent on discharge of epinephrine because it has been observed to occur in adrenalectomized animals, not only in these experiments but also by Cori and Cori (16) and Bridge (9).

Because of the lengthy survival of the adrenalectomized animals used by Cori and Cori, the participation of the adrenal cortex was conceivable. However, the experiments of Bridge and of this paper exclude this possibility.

Hypoglycemia affecting the liver directly would appear to be ruled out by the present experiments.

On comparing groups 2 and 3, it would be possible to say that the decreased liver glycogen was accounted for by the increased muscle glycogen, by the increased oxidation which is reflected in the increase of unaccounted carbohydrate, or by a combination of the two; when, however, the figures for groups 3 to 6, inclusive, are considered, it is not easy to make out any certain tendency for a progressive increase in either as the insulin dose is increased. Furthermore, merely to account for the glycogen lost from liver as being disposed of elsewhere in no wise minimizes the fact that it *has* decreased and has done so in the face of a hyperglycemia which does not decrease with progressive increases in the amount of insulin employed. In group 7 glucose alone as in group 2 was given for 2 hours, followed, however, by an extra hour of the same glucose injection plus insulin. At the end of 2 hours it is to be supposed that liver glycogen was at the level of group 2. In the third hour despite the extra sugar injected, and which

sustained the blood glucose level, the liver glycogen dropped sharply under the influence of insulin.

As regards the possibility that insulin by reducing gluconeogenesis from protein could explain the effect, it should be pointed out that if all protein catabolism were stopped (75 mgm. urinary nitrogen per 100 grams per day, Evans (7)), only 24 of the 74 mgm. drop in liver glycogen (groups 2 and 5) could be accounted for. In addition, neither the explanation of decreased gluconeogenesis from protein, or from fat as argued for by Soskin, could account for the fact that liver glycogen has not been deposited during hyperglycemia.

In table 3 of the paper by Cori and Cori (11), for rats fed glucose and glucose + insulin, the average respiratory quotients are 0.884 and 0.903, respectively. Russell (15) records values of 0.857 and 0.887 for similar experiments. Despite the criticism of the respiratory quotients again raised by Soskin (5), it is difficult to conceive of any rapid and considerable conversion to fat occurring without a sharper rise in the respiratory quotient; it can be calculated that per 100 grams of rat, a conversion of 50 mgm. of carbohydrate to fat in 1 hour would result, other things being equal, in a rise of approximately 0.100 in the respiratory quotient. Further, to explain the fall in liver glycogen under the action of extra insulin as due to conversion to fat still leaves unexplained the failure to deposit liver glycogen from circulating glucose.

The experiments of Corey and Britton (17) on glucose-perfused liver and of Ostern, Herbert and Holmes (18) on glucose-bathed liver brei give some indication that the insulin effect here discussed may be directly upon the liver.

It may be that the findings of this paper represent the pharmacologic and not the physiologic action of insulin upon the liver; in this connection it is to be noted that the rat is not very insulin sensitive and that the smallest doses used do not greatly exceed on a weight basis the amounts occasionally used clinically; furthermore, because the experiments were short and relatively acute, the maximal effect of the insulin was probably not achieved. Should the effect be a pharmacologic one, it is nevertheless highly specific and as such is of interest.

Crystalline insulin (Lilly) was used in four of the experiments of group 5, and the results could not be differentiated from those due to ordinary insulin solution.

The experimental results of this paper do not justify an extended discussion of the perplexing problem of insulin action and the awkward facts connected with it; these have been dealt with in the competent reviews already mentioned. It is, however, appropriate to emphasize the fact that *a*, insulin lowers liver glycogen in the intact animal; *b*, progressively so with increase of insulin dosage; *c*, without mediation of the adrenal,

and *d*, despite hyperglycemia; it is also argued that *e*, suppression of gluconeogenesis or increased conversion to fat does not explain non-deposition of liver glycogen. The evidence tends to indicate that the effect is directly upon the liver.

SUMMARY

Rats were injected intravenously with glucose with or without insulin.

Cardiac glycogen was found to be increased by glucose, but not further increased by the addition of insulin.

For such glucose injected animals, the liver glycogen was found to be lowered by insulin and progressively so as the dose was increased; the effect occurs even during hyperglycemia; it is not mediated by the adrenal. Possible explanations are discussed.

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INHIBITION OF THE PYLORIC SPHINCTER REGION BY THE DIGESTION PRODUCTS OF FAT[1,2]

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It has recently been shown by Quigley, Werle, Ligon, Read, Radzow and Meschan (1) that the presence of natural fats (cream or egg yolk) in the duodenum leads to inhibition of the entire pyloric sphincter region. Their evidence indicated that the well known delay which fats exert on gastric evacuation resulted from the cessation of antral propulsive peristalsis and occurred in spite of the relaxed pyloric sphincter and bulb. Whether this action resulted from neutral fats *per se*, from their digestion products or from both groups of compounds has not been determined. Tönnes and Nevers (2) claimed that neutral fats in the duodenum did not modify gastric evacuation and therefore only the digestion products were effective. Their report cannot be accepted for they employed no method to prevent digestion of the neutral fat they administered. Card's (3) demonstration that a variety of fat digestion products inhibited the body of the stomach is only indirectly applicable to the question of gastric evacuation.

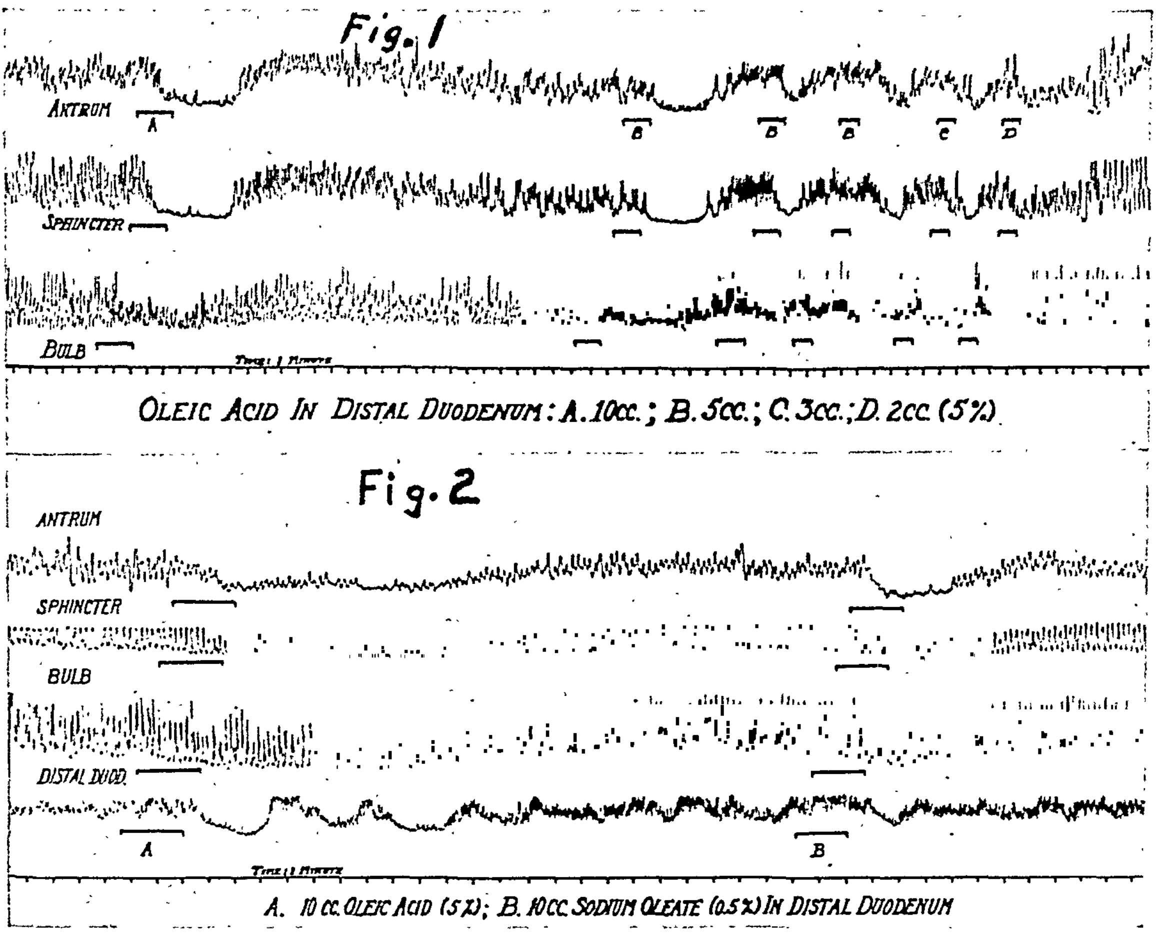
The present study constitutes the first investigation of the effects of pure fat digestion products on the motility of the sphincter region. We recorded motor activity from balloons placed in the pyloric antrum, sphincter, duodenal bulb and distal duodenum of 7 trained dogs provided with permanent cannulae opening into the stomach and duodenum (method of Meschan and Quigley (4)). The test solutions, at body temperature, were slowly introduced 18 hours post cibum into the duodenum just beyond the distal duodenal balloon.

Fatty acids. The following substances were administered in 10 cc. quantities of 5 per cent emulsions in acacia; oleic acid (40 expts.), linoleic acid (6 expts.), palmitic acid (3 expts.), ricinolic acid (8 expts.), myristic acid (5 expts.). Each of these acids inhibited the entire pyloric sphincter

[1] A preliminary report of this work was made in the Review of Gastroenterology 4: 272, 1937.

[2] This investigation was supported in part by a research grant from the Ella Sachs Plotz Foundation.

region (fig. 1) in a manner similar to that of the natural fats. The various fatty acids always produced a decrease in frequency and magnitude of the recorded waves but displayed some quantitative variation in action. This inhibition was progressively less complete in passing from the antrum to the distal duodenum and began in 75 to 120 seconds. Maximal inhibition



Registration by the tandem balloon-water manometer technic.

Effects on the pyloric sphincter region of fat digestion products introduced into the distal duodenum.

Fig. 1. Effects of varying quantities of oleic acid.

Fig. 2. Comparison of effects from 10 cc. 5 per cent oleic acid with those from 10 cc. 0.5 per cent sodium oleate.

developed in about 3 minutes followed by complete recovery after approximately 10 minutes. A repetition of effects from a series of small doses was also demonstrated (fig. 1). The effect from 10 cc. of 5 per cent oleic, or linoleic acid was comparable to that from 4 cc. of cream (30 per cent B.F.), thus these free fatty acids exhibited about twice the inhibiting action on the sphincter region shown by the natural fat. Palmitic, ricinoleic and

myristic acids were less than one-half as effective as the other acids in this series (i.e., they were about as active as the natural fats). The inhibition from these three fatty acids, especially ricinoleic acid, frequently was less complete than from cream, and of approximately half the duration. The latent period was shortest with myristic acid (1 min.) and longest with ricinoleic acid (8 min.). The injection of 20 cc. quantities of the fatty acid emulsions increased only the duration of the inhibition and this increase was moderate.

Soaps. Sodium oleate, 10 cc. 0.5 per cent (15 expts.), sodium myristate 10 cc. 0.5 per cent (5 expts.), and sodium palmitate 10 cc. 2 per cent (4 expts.) produced marked inhibition of the sphincter region (fig. 2), but the depression was less complete and of only half the duration of 10 cc. of cream. Sodium ricinoleate 10 cc. 5.0 per cent (9 expts.) produced only moderate inhibition. The effects from sodium butyrate 10 cc. 5.0 per cent (9 expts.) were variable, with some tendency toward augmentation of bulbar activity. The early effect of sodium myristate on the bulb was augmentory, but this was usually followed by an inhibition.

Placed in order of their decreasing effectiveness, the compounds in our series were oleate, myristate, palmitate and ricinoleate. This order applied to the free acid or to the sodium soap. The soaps appeared to be approximately ten times as effective as the free fatty acids, but the doses employed did not give sufficiently comparable results to permit a strict comparison.

Emery and Edwards (5) found the irritant action of sodium soaps on the human skin decreased in the following order: myristate, oleate, ricinoleate, palmitate. Thus the sphincter-inhibiting action of soaps probably does not depend on its irritating action. Oleic acid and ricinoleic acid have the same number of carbon atoms and both are unsaturated compounds, but oleic acid is a much more effective depressant of the sphincter region than is ricinoleic acid. Our results afford no confirmation of the report of Roberts (6) that the delay in evacuation is related to the degree of unsaturation of the fat employed.

Glycerol and mineral oil. The administration of 10 cc. quantities of 0.5 to 2.5 per cent glycerol solution (18 expts.) produced variable effects on the sphincter region. All the effects were slight and favored the conclusion that such quantities of glycerol were without specific effect. Similar quantities of 3 to 5 per cent glycerol produced nausea and vomiting.

Heavy mineral oil (7 expts.) was used undiluted in 10 cc. quantities. No alteration in the motility of the sphincter region resulted, thus indicating that the inhibitory actions of fats were not dependent on the physical properties of oils. This was in accord with the observation of Edelman (3) that vaseline in the intestine failed to inhibit gastric evacuation.

Discussion. The inhibition of the pyloric sphincter region following the intraduodenal administration of soaps or fatty acids is qualitatively similar to that produced by natural fats. The action progressively diminishes from the antrum to the distal duodenum. The retardation of gastric evacuation produced by the entrance into the duodenum of fats or their digestion products apparently results from a decreased antral peristalsis and occurs in spite of a relaxed sphincter.

In producing inhibition of the sphincter region, the fatty acids were 1 to 2.5 times as effective as the natural fats. The soaps varied greatly from sodium butyrate which was less effective than cream to sodium oleate and sodium myristate which were approximately 30 times more effective.

Our experiments bear on the question whether fats *per se* inhibit the sphincter region or only become active after digestion, but they do not supply a positive answer. Only a few of the digestion products of fats used in our experiments were more effective than the natural fats, the others were either less active or equal in activity to the fats. Moreover, the latent period of 75 to 100 seconds was the same with either fats, fatty acids or soaps. If only fat split products were active, neutral fat could only become effective after it had undergone digestion and a significant concentration of the products had come in contact with the mucosa and initiated the influence on the sphincter region. We might assume that under favorable conditions this series of events could occur within the latent period noted for fats. However, the latent period was within these limits even under conditions which should retard the process: *a*, when 30 cc. of cream was employed, the enzyme and digestion products were markedly diluted and mechanically kept away from the mucosa, or *b*, when the pancreatic juice and bile were sucked out of the proximal duodenum and blocked by a balloon from entering the distal portion of the duodenum into which the cream was introduced. Thus our observations favor the conclusion that natural fats initiate the sphincter region inhibition and the action is continued by the fatty acids and soaps formed during digestion, but the glycerine is inactive.

SUMMARY

Studies made with the tandem balloon method demonstrated that the introduction of fatty acids or soaps into the proximal intestine of fasting dogs inhibited the motility of the pyloric antrum, sphincter and duodenal bulb in a manner qualitatively similar to that produced by natural fats. Usually the order of decreasing activity was soaps, fatty acids, natural fats. However, the evidence indicated that if natural fats were administered, they initiated the inhibition and the digestion products continued the influence.

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ROENTGENOGRAPHIC OBSERVATIONS SUGGESTING DIFFERENCE BETWEEN TOTAL AND CIRCULATING BLOOD VOLUME

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Reduction in measured blood volume after various constricting drugs has been reported by various observers, including Freeman (1) after adrenalin, Griffith, Comroe and Zinn (2) after ergotamine tartrate, and Griffith, Corbit, Rutherford and Lindauer (3) after pressor doses of pitressin. In the latter study in a series of 10 rats blood volume, as measured by the dye method of Griffith and Campbell (4), was found to be so greatly reduced that the results were scarcely credible.

If the peripheral capillaries of the rat's foot are observed in such an experimentally treated animal it is seen that, as a rule, blood cells remain in the capillaries but the flow stops. As this observation could apply only to a limited number of vessels of a peculiar type, the skin capillaries, it was decided to extend it to larger vessels using intravenously injected thorotrast and x-ray. Moreover, we planned to follow blood volume changes by other methods,—by hematocrit, hemoglobin and plasma protein determinations.

METHOD. A. *Observations of the general vascular tree of the rat by x-ray.* Large albino rats were used, usually weighing 300 grams or more. Under nembutal anesthesia one of the larger veins was exposed, usually one of the femoral veins. A small hypodermic needle was inserted and an amount of blood was withdrawn which was intended to equal the amount of thorotrast which was to be given, usually 3 cc. It was not always possible to withdraw quite this much blood. However, in preliminary experiments when blood was not withdrawn it appeared that there was little difference in the vascular shadows, although the animal in general was not in as good condition. Immediately after the injection of thorotrast, fluoroscopic observation was begun and, at intervals, roentgenograms were made.

Two procedures were followed: 1. *The thorotrast-pitressin procedure.* The thorotrast was given first and fluoroscopic observations begun. Then

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5 pressor units of pitressin were given intraperitoneally and the fluoroscopic observation continued for 30 minutes. Five animals were used.

2. *The pitressin-thorotrast procedure.* The same dose of pitressin was given, followed in 30 minutes by the thorotrast. Then fluoroscopic observation was continued for 20 to 30 minutes. Several animals died shortly after the intravenous injection of thorotrast, but five survived and appeared to be in satisfactory condition.

B. In 5 normal albino rats hemoglobin, hematocrit, and plasma protein measurements were made immediately before and 30 minutes after the intraperitoneal injection of 5 pressor units of pitressin. The technique for these measurements in rats has been previously reported (5, 6). Dilution or concentration of blood volume is expressed in terms of per cent of the original measurement. The results obtained were compared with those previously reported (3) in which blood volume was measured several days before and again 30 minutes after giving the pitressin. At the time of each measurement vital red was injected into the jugular vein and its concentration measured in heart blood obtained 4½ minutes later.

RESULTS. A. *Thorotrast-pitressin procedure.* The artery and corresponding vein show as a single vascular shadow. The general vascular bed was well outlined by the thorotrast, including vessels to the extremities. After the injection of pitressin there was an abrupt decrease in the size and increase in the rate of the heart, occurring within a few seconds, with little other appreciable effect.

Pitressin-thorotrast procedure. The heart was somewhat small when first observed. The central vessels of the body, including those of the thorax, abdomen and kidney, were shown as well as or even better than in the previous procedure, but the vessels to the limbs were seen faintly if at all. Ten minutes or more after the injection of thorotrast the vessels of the limbs showed more distinctly but not to the extent seen in animals in the thorotrast-pitressin procedure.

B. Figure 1 shows the result of estimating change in blood volume by various methods. The change in hematocrit reading 30 minutes after pitressin indicated a change in blood volume ranging from an 11 per cent concentration to a 42 per cent dilution, but averaging a 14 per cent dilution or increase in blood volume. The change in hemoglobin indicated an average change in blood volume of only 2 per cent dilution, but the range was from 11 per cent concentration to 28 per cent dilution. Based upon change in plasma protein the blood volume appeared to have increased by 1 per cent, but the range was from 33 per cent concentration to 21 per cent dilution. It should be noted that in all instances the blood for the second sample was secured from the heart, as after pitressin blood cannot be obtained by cutting a tail vein. The preliminary sample was obtained in

2 instances from the heart and in 3 from a tail vein. In normal animals samples obtained by either route are essentially identical.

These figures must be compared with those previously obtained (3) using vital red. This method indicated an average reduction of blood volume by 38 per cent, ranging from 10 to 60 per cent.

Discussion. It is known that blood cells may remain for considerable periods within the minute vessels of the spleen, liver, and bone marrow without entering into the general circulation. However, they may enter into the general circulation, either gradually or suddenly, when physiologic need arises. Some plasma must also be retained with the cells, but

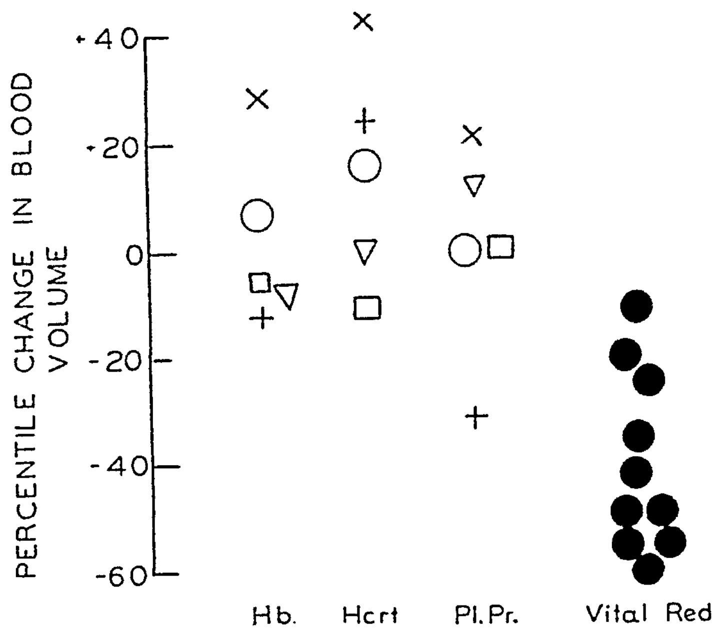

Fig. 1. Percentile change in blood volume after pitressin estimated in ten rats (●) by repetition of vital red blood volume determination and in five other rats, each designated by a particular symbol, by each of three methods, change of hemoglobin, of hematocrit, and of plasma protein concentration.

it is uncertain whether the amount is as great as though the cells were actually circulating. Blood retained in such vascular reservoirs would not be included in measurements of blood volume made by a dye method. If, for any reason, more of the circulating blood was withdrawn into such reservoirs, provided both cells and plasma were withdrawn proportionately, blood volume as measured by a dye method would be greatly decreased but the blood volume as estimated from hematocrit, hemoglobin or plasma protein measurements would be unchanged. It is conceivable that, under conditions of intense vasoconstriction, circulation to certain parts or organs may be arrested to the extent that they, too, serve as reservoirs for non-circulating blood. Even if the blood in such parts or organs does

circulate, but very sluggishly, the mixing time for dye used in blood volume measurements may be so greatly increased over the normal that, if the interval for the normal be used, all figures obtained will be too low.

To recapitulate, under conditions of vasoconstriction, fluid may be lost from the blood, resulting in some degree of hemoconcentration and lowered blood volume. Such a lowering of blood volume could be demonstrated by serial determinations of hemoglobin, hematocrit values, plasma protein, or by a dye method for measuring blood volume. On the other hand, if vasoconstriction results in trapping of blood in areas without active circulation, changes in blood volume will be estimated as slight if based on hemoglobin, hematocrit, or plasma protein measurements, but great if based upon a dye method, *since this measures circulating* blood volume rather than total blood volume. This latter phenomenon appears to have occurred in the experiments here recorded.

SUMMARY

After a strong vasoconstricting agent, pitressin, blood volume measured by a dye method is greatly reduced. We suggest that a considerable amount of blood may be trapped in areas of the peripheral circulation so that actively circulating blood volume may be much less than total blood volume. The blood still circulating shows relatively slight changes in hematocrit red cell volume or in plasma protein concentration. The presence of blood vessels in the extremities containing blood but without active circulation is shown by the following: 1. By microscopy, the skin capillaries contain red cells, but there is no flow. 2. Thorotrast introduced into the general circulation before giving the pitressin remains in and outlines the vessels of the extremities. 3. Blood cannot be secured by cutting a tail vein. 4. Thorotrast introduced into the general circulation after the injection of pitressin either does not enter into and outline the vessels of the extremities or does so tardily and to a lesser extent.

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